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ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO.	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci. Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

PHYSIOLOGY

REFLEX CONTROL OF CEREBRAL CIRCULATION

COMMUNICATION III. THE ROLE OF THE CAROTID SINUS AND THE INTEROCEPTORS IN THE REGULATION OF CEREBRAL CIRCULATION

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(Received April 8th, 1957. Presented by Active Member of the Academy of Medical Sciences, USSR, V.N. Chernigovsky)

In spite of many investigations, the problem of the effect of the sinocarotid reflexes on cerebral vascular tone remains unsolved. According to Bouckaert and Jourdan [4], A.A. Shlykov [3], K. Gollwitzeer-Meier [5], and N.M. Ryzhova [2] and others, stimulation or excision of the sinocarotid receptors causes expansion or contraction of the cerebral vessels independently of changes in the arterial pressure. However, H. Rein [10] M. Schneider and D. Schneider [11], C. Heymans [7] and others found that these cerebral blood vessel changes are accompanied by changes in the arterial pressure, and deny that impulses from the carotid sinus have any direct effect on the blood vessels.

In our laboratory N.M. Ryzhova has shown that these contradictory findings are due in many cases to faulty methods. She showed that under normal conditions, impulses from the baroceptors of the carotid sinus maintain the cerebral vessels in a somewhat dilated condition. Increase of impulses causes a further dilatation, while their elimination causes a marked contraction of these vessels. From this, one would expect that in pressor and depressor reflexes, a change in the number of impulses from the carotid sinus would affect cerebral vascular tone. However most authors maintain that this mechanism plays no part in the reflex control of cerebral circulation, but claim that the control is mediated through changes in arterial pressure (C. Heymans [7], B. N. Klosovsky [1] and others).

In studying changes in cerebral circulation due to stimulation of the intestinal receptors, we showed that the observed changes in the blood flow through the cerebral vessels are caused not only by changes in the arterial pressure, but also by changes in the size of the lumen of the vessels as they contract or expand. The rate at which the size of the lumen changes after stimulation indicates a direct nervous action. After considering the parallel changes in arterial pressure and flow rate during stimulation of the interoceptors in normal conditions and in hypercapnia we concluded: 1) there is a reflex control of vasoconstrictor and vasodilator nerves; 2) whichever of these two effects prevails, resulting in a contraction or a dilatation of the cerebral vessels, depends to a considerable extent on the initial tone of the cerebral vessels.

Because the impulses from the sinocarotid receptors exert a considerable control over the tone of the vessels in normal conditions, and are increased or decreased by changes in the arterial pressure, it is probable that the carotid sinus controls reflex changes in cerebral circulation.

EXPERIMENTAL METHODS

To confirm this hypothesis, first, we reduced the flow of impulses from the baroceptors of the carotid sinus by applying pressure to the carotid artery, and took measures to ensure that any increase in arterial pressure would not cause an increase in this flow, then, we found the effects of stimulating the (intestinal) interoceptors on the blood flow through the vessels of the cerebral meninges. An inelastic cuff of soft lead 4-7 mm thick was placed around the bifurcation of the carotid artery so that it enclosed this region without exerting pressure on it and without restricting the blood supply to the brain, but, so that it prevented any stretching of the vessel walls, occurring upon increase of arterial pressure. This method was based on that of C. Heymans [7], E. Koch [8], W.H. Hauss, H. Kreuziger and H. Asteroth [6], and S. Landgren [9], who showed that the stimulus to the baroceptors is the stretching of the walls of the sinus and not the pressure itself within the sinus. In a separate investigation it was shown that increase of pressure within the sinus causes no increase in the flow of impulses in the nerve from the sinus or any fall in arterial pressure.

The experiments were carried out on 25 dogs under morphine-urethane anesthesia. Simultaneous recordings were made on a photokymograph of the arterial pressure – using a membrane manometer in the femoral artery, of the respiration – using a pneumograph,of the blood flow in the cerebral meninges – using a thermoelectric method with a fine thermoelectrode (A.M. Blinova and N.M. Ryzhova). Stimulation of the interoceptors was effected by introducing air at a pressure of 80 – 90 mm into a section of small intestine.

EXPERIMENTAL RESULTS

As we showed previously, stimulation of the interoceptors of the intestine in anesthetized animals causes an increase of arterial pressure and an increase in blood flow through the vessels of the cerebral meninges, in most cases, though the effect varies somewhat from one animal to another. Compression of the carotid arteries causes an increase in arterial pressure and a reduced blood flow to the brain. In certain cases,after clamping the carotid arteries, stimulation of the interoceptors causes a poorly shown increase in blood flow to the brain, or else none at all. Fig. 1 shows the results of one of these experiments. Stimulation of the interoceptors caused first an insignificant fall and then a small rise in arterial pressure and a well-shown increase in cerebral blood flow, which at the end of the stimulation gradually returned to the original value (Fig. 1,a). When the carotid arteries were clamped and the same stimulus applied, there was a very much smaller and poorly-maintained increase of blood flow, despite the large increase of arterial pressure (Fig. 1,b). In most of these experiments with clamped carotid arteries stimulation of the interoceptors had an effect on cerebral blood flow which was the reverse of the normal reaction.

In the experiment shown in Fig. 2, in the first reaction there was a well-marked increase in arterial pressure and a small increase in cerebral blood flow (Fig. 2,a). When the carotid arteries were clamped, stimulation of the interoceptors, instead of causing an increase, caused a marked reduction in blood flow (Fig. 2,b). The increase in the arterial pressure was even greater than in the first reaction, so that the depression in the blood flow shows a considerable increase in tone, i.e. contraction of the cerebral vessels.

After placing the cuffs on the point of bifurcation of the carotid arteries, stimulation of the interoceptors did not cause a reversed reaction on the cerebral vessels. But in all these experiments we found that after stimulation there was a smaller increase in blood flow to the cerebral vessels than in the first reaction (prior to putting on the cuffs).

Figure 3 shows the results of one of these experiments. The increase in cerebral vessel blood flow which is observed before placing the cuffs in position and after stimulation of the interoceptors (Fig. 3,a) no longer occurs when the stimulation is applied with the cuffs in position (Fig. 3,b). When the cuffs are removed the original reaction is again restored, and in some experiments the effect is even greater than after stimulation prior to putting on the cuffs (Fig. 3,c).

Thus, when increase in baroceptor impulse flow following increased intrasinus pressure is prevented, the reflex leads to a smaller dilatation of the cerebral vessels than in the case with normal carotid sinus function. After elimination, by clamping the carotids, of the impulses from the carotid sinus baroceptors which serve to dilate the cerebral vessels, the reflex action results in a contraction instead of the usual dilatation of the cerebral vessels, in most cases. These observations show that the more strongly the vasoconstrictor action at the

time the interoceptor stimulation occurs the fewer the vasodilator impulses from the carotid sinus receptors, i.e. the stronger the tone of the cerebral vessels.

The greater effects of interoceptor stimulation with clamped carotid arteries may follow from two causes. First, when the stimulus is applied, there is already an increased cerebral vessel tone, so that the same arterial pressure cannot cause a dilatation on account of the increased firmness of the walls (E. Wetterer and H. Piper [12]). Second, the dilator action of the impulses from the aortic region is not sufficient to compensate for the absence of the impulses from the carotid sinus, as the aortic mechanism has already been expended in compensating for the absent carotid sinus impulses caused by the reduced pressure following clamping the carotids. The greater increase of arterial pressure during stimulation of the interoceptors with clamped than with unclamped carotids, also points to the inadequacy of the aortic mechanism. At the same time, after the cuffs have been applied, the function of the aortic mechanism is still intact. Under these conditions there is no greater increase in arterial pressure than there was initially, as the absence of an increased flow of impulses from the carotid sinus baroceptors is completely compensated for by the increased flow from the baroceptors of the aortic region. However the smaller increase in cerebral blood flow during the interoceptor stimulation indicates the inadequacy of the compensation as affecting dilatation of the cerebral vessels.

The results we have obtained allow us to draw the following conclusions: the impulses from the baroceptors of the carotid sinus during pressor interoceptor reflex action reduce cerebral vessel tone and so increase the blood supply to the brain; reduction of the elasticity of the arterial wall of the carotid sinus may have the unfavorable effect of causing a reduction in cerebral blood flow following interoceptor stimulation.

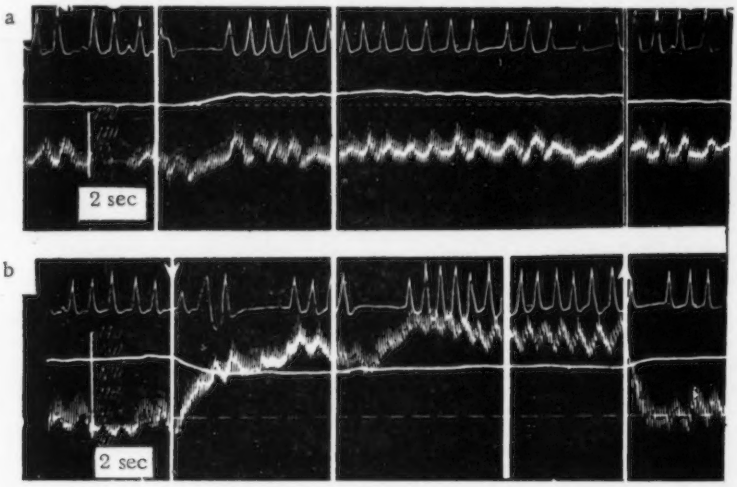

Fig. 1. Changes in cerebral blood supply after elimination of baroceptor impulses. Experiment of January 13, 1956. Curves, from above downward: Respiration; blood flow in cerebral meninges; arterial pressure. a) Initial reaction; b) reaction with clamped common carotid arteries. Vertical lines) beginning and end of stimulus; double vertical lines) interruption of record; vertical lines with an arrow) application and removal of clamps to common carotid arteries. Dotted line) values of the recorded functions prior to experiment. Traces read from right to left.

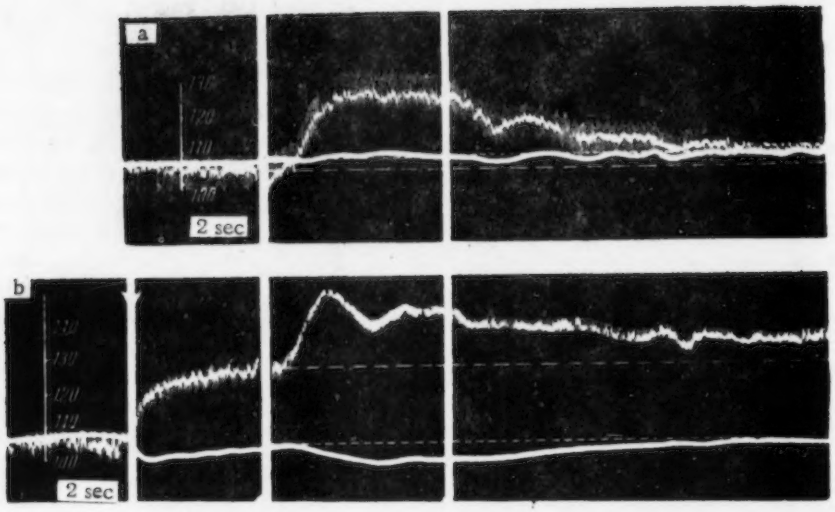

Fig. 2. Changes in cerebral blood flow. Experiment of June 6, 1956. Curves, from above downward: Blood flow in the cerebral meninges; arterial pressure.
a) Initial reaction; b) reaction after clamping common carotid arteries. Significance of vertical and dotted lines as in Fig. 1. Traces read from left to right.

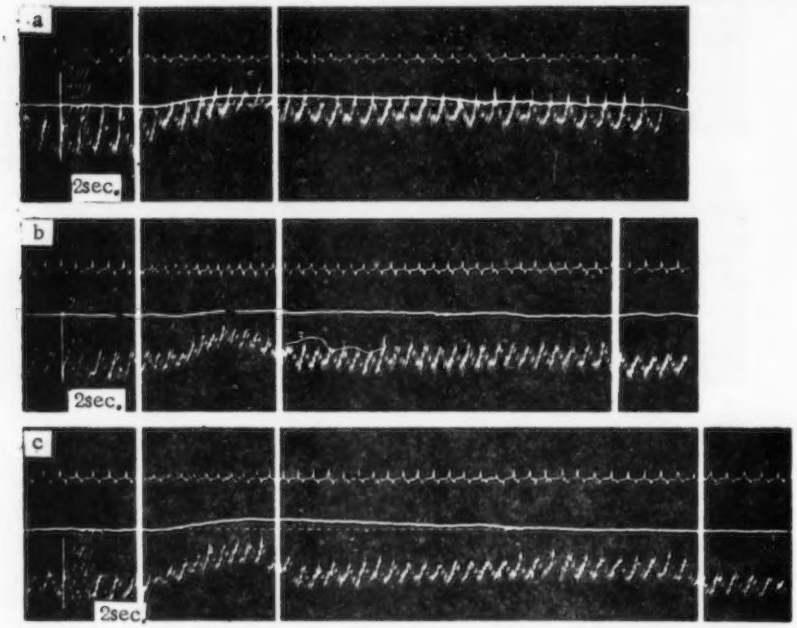

Fig. 3. Changes in cerebral Blood supply. Experiment of Feb. 28, 1956. Curves, from above downward: Respiration; blood flow to cerebral meninges; arterial pressure.
a) Initial reaction; b) reaction with cuffs on both carotid sinuses; c) reaction 15 minutes after removal of the cuffs. Significance of vertical and dotted lines as in Fig. 1. Traces read from left to right.

SUMMARY

The change of the volume velocity of the blood circulation was studied in the brain meninges during stimulation of intestinal interoceptors, which caused hypertension and increased the blood circulation of the brain. The thermoelectric method was employed. In stimulation of interoceptors with occluded carotid arteries there was a decrease in volume velocity of the blood circulation of the brain, instead of increase. When cuffs were placed on the area of carotid bifurcation the increase of the volume velocity was less marked than in the original reaction (before the application of the cuffs). It was concluded that the impulses from the baroreceptors of the sinocarotid zone during the pressor interoceptive reaction cause decrease of the tonus of the brain vessels and provide increased blood supply to the brain.

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* In Russian.

** Original Russian pagination. See C. B. Translation.

PROPRIOCEPTOR REFLEX CARDIAC EFFECTS

PROPRIOCEPTOR REFLEXES AFTER SECTION OF THE POSTERIOR COLUMNS

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It is known that the afferent pathways associated with motor control pass through the white matter of the posterior columns of the spinal cord. Various investigators [1,2,3,4,5,6], who have attempted to explain the mechanism of motor conditioned reflexes, have damaged the white matter of the posterior columns and then made observations on these reflexes after motor control had been re-established.

At present two contradictory views are held. I.M. Apter [1] and V.P. Protopopov [5] found that after section of the posterior columns previously elaborated conditioned reflexes disappeared and failed to recover. On the other hand, L.S. Gambaryan [2,3,4] maintains that not only section of the posterior columns but their total destruction over several segments does not destroy previously elaborated conditioned reflexes or prevent the formation of new ones.

It must be noted that these authors did not study proprioceptor reflexes. They investigated the motor reaction in response to electrical stimulation or to food offered by hand. Consequently, there is no foundation for the conclusion based on the elimination of the afferent pathways from the muscles and joints of the limbs [3] as in these cases the motor reaction is adequately mediated by the lateral and anterior columns.

I.M. Sechenov [7] and M. Schiff [9] in experiments on frogs, showed that after section of the posterior columns, proprioceptor impulses may pass through the damaged portion; this is possible because of the internuncial connections of the grey matter of the spinal cord.

This has been confirmed by E.D. Gardner and F. Morin [8] in experiments on apes, using an electrophysiological method. Here it was found that after section of the posterior columns, the passage of proprioceptor impulses to higher levels of the central nervous system was greatly impaired.

EXPERIMENTAL METHODS

In one of the dogs, 14 months after section of the posterior columns between C_2 and C_3,* we investigated

* A histological investigation carried out at the end of the experiments showed that there were very considerable changes in the posterior columns of the spinal cord. At the site of the section there was a fine scar which stained dark red with picrofuchsin. Above the scar in the region of the posterior columns the myelin coats and axis cylinders of most of the nerve fibers had disappeared. In places where the nerve fibers had disappeared, a finely fibrous tissue could be seen, which gave a negative reaction for myelin. (The investigations were made in the Department of Pathological Anatomy with the cooperation of D.S. Sarkisov).

the effect of proprioceptor action on the heart, using the method described by us previously. Before the operation the animal had become familiar with the bench on which it stood, and had been trained to support on its back for 10 minutes a load equal to its own weight, and it was not until the end of this period that it began to shift its weight from one foot to another and to whimper. Fourteen months after the operation, there was almost complete compensation of motor function, externally. But even then, the animal would sometimes stand for long periods on the dorsal surface of the paw. It was unable to support a load equal to its own weight, and fell being unable to rise with the load. The animal could support a load of 80% of its own weight for not more than 3-4 minutes, and could stand quietly with this load for only about 2 minutes. This load of 80% of the body weight applied for 2 minutes was then used as an unconditioned stimulus. Positive conditioned stimuli: light (60 w electric lamp) and a metronome giving 120 beats per minute (M_{120}), and as a differentiated stimulus a metronome at 60 beats per minute was used (M_{60}).

EXPERIMENTAL RESULTS

In the first experiments a static load by itself caused a considerable change of heart rate which lasted from the time the load was applied until 12-15 minutes after its removal; however, after the sixth trial this effect occurred only during the action of the unconditioned stimulus. This change became very small and was sharply augmented only when the animal moved with the load. The static effect had disappeared. No acquired skill in carrying the load could be observed.

In unoperated animals before conditioned reflexes were formed, the stimuli of light, M_{120}, M_{60}, and bell caused changes in heart rate only in 1-4 tests with the isolated stimuli, and after that were ineffective. After removal of the posterior columns these stimuli caused a considerable increase in heart rate and changes in the electrocardiogram both of which lasted for quite a long time. The reaction of the heart associated with the orientating reaction of the animal to the bell remained, and was not extinguished after repeated tests with this stimulus over a period of 4 months. The following differences were also noted (see Table).

TABLE

Changes in the Conditioned Reflexes in Dogs after Section of the Posterior Columns of the Spinal Cord caused by Operation

Conditioned reflex	Number of combinations	
	in unoperated healthy dogs	in dogs after section of the posterior columns
Formation of conditioned reflex to light	—	18-25
Formation of conditioned reflex to M_{120}	5-10	25-36
Extinction of the conditioned reflex to M_{120}	3-4	19
Re-establishment of conditioned reflex to M_{120}	1-2	21
Development of differentiation to M_{60}	1	6
Alteration of the significance of a pair of conditioned stimuli.	3-4	30

From the results shown in the table it can be seen that the conditioned reflex effect on the heart is formed earlier by a weak stimulus (light) than by a strong one (M_{120}).

In the unoperated animals, M_{120} begins to cause changes in heart rate after 5 combinations of the conditioned and unconditioned stimuli, and by the 10th combination it is completely effective. In dogs with section of the posterior columns changes in the heart rate can be induced by the M_{120} alone after 25 combinations though the conditioned reflex continues to grow after 36 combinations (Fig. 1). The differentiated stimulus applied to the unoperated animals caused changes in the heart rate and a broadening of the QRS complex after only 1-4 times. After section of the posterior columns, the same stimulus caused changes in the heart rate which occurred in two phases: when first applied there was an increase in heart rate, and when applied subsequently there was a decrease; differentiation was established after 6 applications of the M_{60} stimulus.

The extinction of the proprioceptor conditioned reflex effect on the heart in dogs after section of the posterior columns takes place 5-6 times more slowly than in nonoperated animals, and its restoration takes place in a manner which is similar to that of forming the conditioned reflex for the first time (Fig. 2).

Simultaneous alteration of the significance of a pair of conditioned stimuli takes place in unoperated animals after 3-4 trials of the stimuli having an altered significance, but takes place differently according to the phase of the training • to carrying the static load: if it occurs in phase IV, a neurosis develops, while if the conditioned reflexes are altered in phases II and III there is no change in the background level of the heart rate. In the operated animals recognition of the altered significance of a pair of conditioned stimuli requires a period 7-10 times longer than in controls, and at a certain period in the alteration there is a considerable change in the background level of the heart rate (Fig. 3).

These results show the great difference between the effects of proprioceptor impulses on the heart in the two groups of animals.

The long time for which the reaction of the heart associated with the orientation reaction of the animal is preserved, and the more rapid formation of a conditioned reflex to a weak stimulus than to a strong one, may be explained in terms of a reduction in tone of cortical cells which results from the elimination of the stream of impulses falling on them from the receptors in the muscles. The less well-shown unconditioned reflex reaction on the heart of the static load, applied to the animal standing still on the bench, and the considerable associated change in the properties of the proprioceptor conditioned reflex effect on the heart indicate an interference in the transmission of impulses, after section of the posterior columns.

The marked and prolonged changes in the heart rate which occur when the static load alone is applied, may be ascribed to changes in the orientation reaction of the animal to this stimulus. At the same time however, in view of other facts, we may suppose that in normal animals the motor apparatus plays a part in the reaction of the animal to the various stimuli in the surrounding medium, and the resulting stimulation of the proprioceptors exerts a correcting effect on the work of the heart.

The absence of the phenomenon of "static effort" and training phases in animals after section of the posterior columns is one more indication that Lindhardt's phenomenon and its changes during the training process are due primarily to the reaction of the central nervous system, conditioned by stimulation of the proprioceptors.

Proprioceptor action on the heart is not completely excluded by high section of the posterior columns.

This may be due to internuncial connections in the grey matter and to spinocerebellar pathways in the lateral columns of the spinal cord. However in normal animals the posterior columns of the spinal cord are the chief pathways along which the proprioceptor impulses which are responsible for the changes in cardiac activity travel to the brain.

• Details of the phases of training to carrying a static load have been given by us in the Byull. Eksptl. Biol. i Med., 1957, 43, No. 1. 14-20.

In our experiments the static load was inevitably associated with stimulation of the tactile receptors over a wide area of the skin of the feet. Experiments on dogs with a transection of the posterior columns of the spinal cord, which leaves the spinothalamic tracts containing some of the tactile fibers intact, may indicate that changes in heart rate in normal animals in response to the application of a static load, are due chiefly to proprioceptor and not to tactile stimulation.

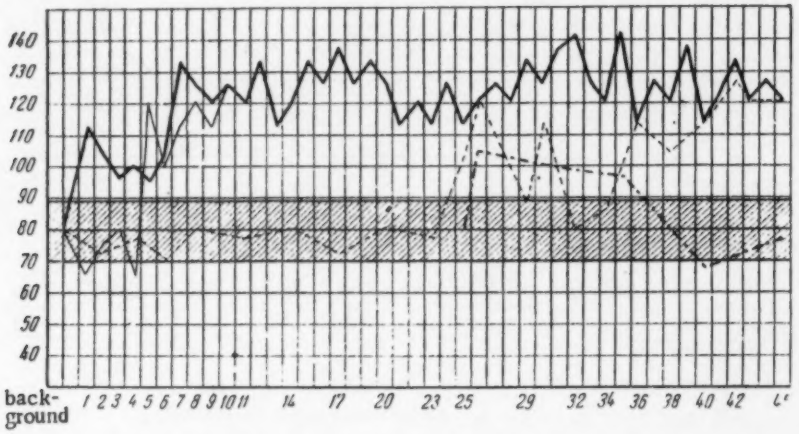

Fig. 1. Formation of conditioned reflexes. Vertically) heart rate, horizontally) number of coincidences of the stimuli. Changes in the heart rate under the influence of: ——— combination of static load with M_{120}, -------- M_{120} alone, – – – M_{60}, —·—· M_{120} in a normal animal. Shaded area) variation of heart rate in absence of stimuli.

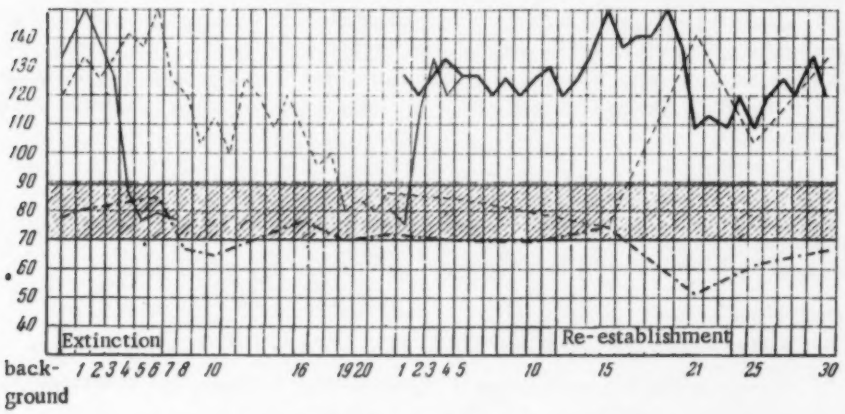

Fig. 2. Extinction and reformation of conditioned reflexes. Traces as in Fig. 1.

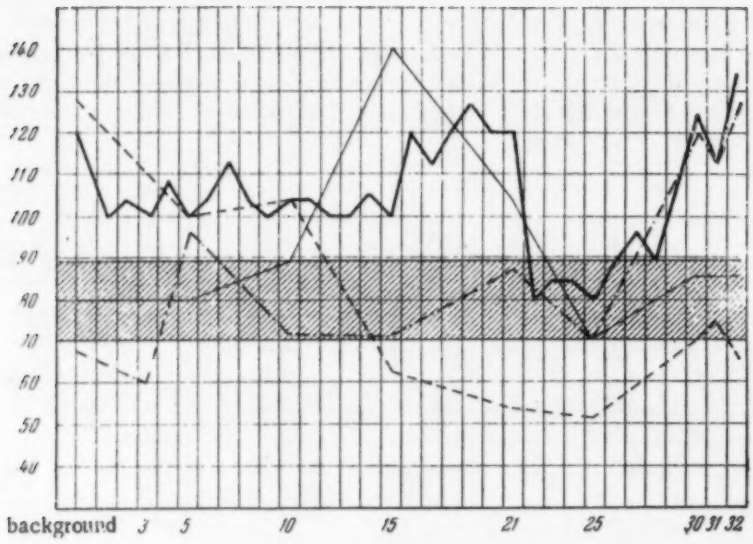

Fig. 3. Alteration of the significance of a pair of conditioned stimuli. Traces as in Fig. 1; ——— combination of static load with M_{80} shaded area -- variation in the heart rate before the alteration, ——— changes in the background level of the heart rate during the process of alteration of the conditioned reflexes.

SUMMARY

The following phenomena were noted in dogs 14 months after section of the posterior trunks of the spinal cord in comparison with intact animals: the value of the time of retaining of the load decreases, the effect of unconditioned reflexes from the proprioceptors on the heart decreases, Lindhardt's phenomenon and training to static load are absent. However, the orientation reaction of the heart to light and sound stimuli is retained for a long time. Formation, extinguishing, restoration and change of conditioned reflexes are 4 to 10 times slower.

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AMYLOLYTIC ADAPTATIONAL CHANGES IN MAMMALIAN SALIVA

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Until now it has not been clear why the saliva in some animals has a very high amylolytic activity, while in others it has practically none (G. Sticker, 1899 [5]; D.A. Biryukov, 1935 [1]).

Some light has been thrown on this as yet unsolved problem by investigations in which the effect of a carbohydrate and a protein diet on the amylase content of human and canine saliva has been studied. In cases where amylolytic activity is high on a mixed diet, there is a noticeable increase or decrease on changing to a carbohydrate or a meat diet respectively. If on a mixed diet there is no amylase in the saliva, then none is found on a protein diet either, but on returning to a carbohydrate diet even on the 4-7th day amylase is found in considerable quantities [2,3,7]. These results help to explain why amylolytic activity is poorly-developed in the saliva of carnivores and well-developed in many herbivores.

It is possible to make one further deduction, whose confirmation is the subject of the present work. Most physiological investigations on this subject are to some extent unsatisfactory, as the tests for amylolytic activity were carried out on only one of the many possible substrates, namely starch. Generally, at any rate as far as phosphorylase is concerned (J. Sumner and G. Somers [8]), this enzyme is completely specific both for starch and glycogen.

In a previous communication [4] we showed that canine gastric juice taken after feeding meat, causes a greater breakdown of animal than of plant protein, while juice secreted by the same animal when fed bread, on the contrary is more effective in hydrolyzing protein of plant than of animal origin. (We have referred to the breakdown of substrates of animal and plant origin as zoolytic and phytolytic activity, respectively.) It remains to find whether it is possible for glands causing hydrolysis of polysaccharides to show the same kind of adaptation as for gastric glands which cause partial hydrolysis of proteins. Our method has been to compare the extent of the breakdown of starch and glycogen under similar conditions. Evidently, if such an adaptation is possible, then it will be most clearly shown by comparing the properties of the saliva of animals with different natural diets.

EXPERIMENTAL METHOD

Fistulae were placed in the ducts of the parotid and submaxillary salivary glands and the amylolytic activity of the saliva determined in the following animals: 3 monkeys, 1 fox, 3 fox cubs, 8 guinea pigs, 3 rats, and 4 cats. In addition, an examination was made of the saliva from the parotid gland in 24 human subjects fed a normal diet.

In 1950-1953 we determined the amylolytic activity using Wohlgemuth's method (parallel determinations using 0.1% starch and 0.1% glycogen in a phosphate buffer at pH 6.5 or 7.2). Subsequently, simultaneous

determinations were made using the method of Wohlgemuth and Smith-Roe [6]. The latter method is very precise. The amylolytic activity is determined by the reduction of the initial concentration of substrate caused by the hydrolysis, (concentration was measured using a photoelectric colorimeter with a red light filter for starch and a blue for glycogen). As distinct from the classical method, a 0.3% solution of starch was used. The same concentration was also used in the case of glycogen. There was usually a good agreement between the two methods.

EXPERIMENTAL RESULTS

In the saliva from both the parotid and submaxillary glands in guinea pigs, there was a considerably greater amylolytic activity with respect to starch than to glycogen. In the parotid saliva of guinea pigs, the phytolytic activity varied between 320-1280 Wohlgemuth units, while for glycogen the corresponding figures were 160-640 units; in each experiment the second figure was 2-8 times less than the first (in most of the experiment it was 4 times less). In guinea pigs the phytolytic activity of the saliva of the submaxillary glands is also 2-4 times greater than the zoolytic, but the general level of amylase in it is somewhat greater (1280-2560 Wohlgemuth units for starch). Saliva from both the parotid and the submaxillary glands was taken during feeding on beetroot, carrot and grass, and from the parotids during spontaneous secretion. It is interesting to note that the spontaneously secreted saliva which represents the natural state of the gland is more strongly phytolytic than zoolytic.

The amylolytic activity of the parotid secretion in rats is even greater (5000-20,000 Wohlgemuth units for starch). The phytolytic activity in these animals is 2-4 times greater than the zoolytic. The amylolytic activity was determined using the method of Smith-Roe [6], and this confirmed that the phytolytic activity was greater than the zoolytic. This was found both for saliva taken during feeding and that stimulated by pilocarpine.

A marked preponderance of phytolytic over zoolytic activity was also found in monkey saliva (Macaque rhesus). The amylolytic activity of the parotid secretion of these animals varied from 10,000-100,000 Wohlgemuth units for starch, and the submaxillary secretion – between 4000-16,000 units. In most of the experiments the phytolytic activity was 2-4 times greater than the zoolytic. In some cases however neither Wohlgemuth's method nor that of Smith-Roe revealed any difference in the rate of digestion of starch and glycogen. The zoolytic and phytolytic activities were equal or very nearly so. The reason for this is not clear, but the occurrence was by no means exceptional. Any systematic error was excluded by making on each sample a measurement with Wohlgemuth's method and, in two optimal dilutions with Smith-Roe's method.

Thus, in this group, fed on an either exclusively or chiefly vegetable diet, amylase hydrolyzed starch more than glycogen.

We must suppose, that on account of the structure of the molecules of starch (more precisely, amyloses) the latter are attacked in preference to the more branched molecules of glycogen. However, even for the substances we have tested, such a conclusion is doubtful, because firstly, the ratio of phytolytic to zoolytic activity varies within fairly wide limits, and secondly, because in certain cases the zoolytic activity is not less than the phytolytic. All this suggests that besides the quantitative variation of the amylolytic properties of saliva (greater or lesser concentrations of enzyme), qualitative changes are also possible and are shown by a change in the preferential action with respect to starch or glycogen.

Considerable variability in the properties of the enzymes were found in cat and fox saliva, but this occurred in addition to the well-known preponderance of zoolytic action. In fox cubs it was not possible to measure the amylase concentration, because there was not enough saliva to use in concentrations less than 1 in 10, and in greater dilutions no amylolytic activity could be observed. In adult foxes phytolytic activity did not exceed 10 Wohlgemuth units, while the zoolytic varied between 40-80 units.

In cats, the concentration of amylase varied between 20-160 Wohlgemuth units for starch (the latter figure occurred only exceptionally). In most experiments these zoolytic properties exceeded the phytolytic by 2-4 times, more rarely the two activities were equal, and still more rarely the phytolytic activity preponderated.

Thus our results confirmed the previous observations on the low amylolytic activity of cat and fox saliva. But in this low degree of activity it remains qualitatively distinct in its action on starch and glycogen from that of the saliva of herbivores.

The hardest task we encountered was that of classifying human saliva correctly. We give here the variation in amylolytic properties (in Wohlgemuth units) of one of the groups we investigated (see Table).

TABLE

Amylolytic Properties of Human Saliva

Subject	Activity		
	phytolytic	zoolytic	ratio
1	5,120	2,560	2:1
2	2,560	2,560	1:1
3	40,960	10,240	4:1
4	1,024	16,384	1:16
5	1,024	8,192	1:8
6	2,048	2,048	1:1
7	16,384	8,192	2:1
8	8,192	8,192	1:1
9	4,096	4,096	1:1
10	16,384	32,768	1:2
11	32,768	3,192	4:1
12	4,096	1,024	1:2
13	4,096	2,048	2:1
14	4,096	2,048	2:1
15	32,768	8,192	4:1
16	32,768	32,768	1:1
17	16,384	8,192	2:1
18	16,384	8,192	2:1
19	32,768	16,384	2:1
20	4,098	512	8:1
21	32,768	16,384	2:1
22	32,768	8,192	4:1
23	32,768	8,192	4:1
24	4,096	1,024	1:2

Thus from these results alone it can be seen that there are three variants of the relationship between phytolytic and zoolytic activity in human saliva: there can be an equality of the two, or else a preponderance of either one or the other. It is clear that in most cases this difference is far greater than any possible experimental error. Late the same variants were found using the method of Smith-Roe.

Quite apart from the problem of what causes these differences, we were concerned to show that there is apparently a variation in the specific activity of the saliva of different individuals. The properties of the saliva of a single individual may vary within wide limits. The ratio of phytolytic to zoolytic activity depends to a considerable extent, though not entirely, on the type of diet. We carried out three experiments to determine the effect of a change from a mixed to a purely vegetable diet • (in 1953 – 2 people were maintained on a

• The participants in these experiments, students and scientific workers, were fed on an arbitrary vegetarian diet, which excluded meat products and extracts.

vegetable diet for 4 days, and in 1954 – 2 people on the same diet for 5 days, and in 1955 – 3 people on the same diet for 8 days), and in all these experiments a sharp reduction in the zoolytic activity was found, as well as an irregular change in the phytolytic activity, i.e. the reaction was adapted to the quality of the current diet.

Thus, as regards the biological aspect of these results, it appears that the enzyme properties of the saliva of different mammals are adapted to the diet: the action is chiefly phytolytic in herbivores and zoolytic in carnivores. Together with the adaptations characteristic of the species there are also marked individual adaptations (experiments on human subjects). Thus in some cases the saliva preferentially breaks down starch, and in others glycogen. This could be brought about either by the enzyme properties remaining unchanged while those of the medium in which it acts becoming altered, or with changes in the property of the enzyme itself. When it is remembered that the monkey and human saliva for the estimation of amylase was diluted hundreds or thousands of times in buffered solutions, it is clear that variation of the medium in which the enzyme acts would not exceed the limits of experimental error. Therefore preponderance of zoolytic or phytolytic properties of the saliva are likely to be due to changes in the specific activity of the saliva amylase.

SUMMARY

Saliva of herbivorous animals (rats, guinea pigs and monkeys) splits starch (phytolytic effect), more actively than glycogen (zoolytic effect), while the saliva of carnivores (cats and foxes) on the contrary splits glycogen more actively than starch. The specific activity of saliva of man shows wide variations in different individuals. In one group there is a prevalence of zoolytic activity, in another phytolytic, while in certain persons both activities are equal. The data that were obtained are considered to be an adaptive mechanism of the enzymatic activity to the quality of food. The adaptive mechanisms not only common to the same species were revealed but, likewise, those which appear with the change of the diet.

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DEVELOPMENT OF PROPRIOCEPTIVE REFLEXES IN THE MUSCLES OF MASTICATION

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Investigations on the development of reflexes show that the proprioceptor reflexes are among the first to develop [1]. Reflex response to stretching the muscles of the limbs in embryo cats and newborn kittens have been described several times [2,6].

It has been shown that reflexes involving mouth movements are well-developed in the embryo [3,4,5,7, 8], but no precise description of the development of the masticatory reflexes has been given as yet.

The present investigation concerns the tone of the masticatory muscles (m. masseter) and the reflex response to stretching them in animals in the early postnatal period.

EXPERIMENTAL METHODS

The experiments were made on 36 kittens at times from 2-3 hours to 30 days after birth. A cathode ray oscillograph and amplifier [4] was used to record the activity of the masticatory muscles. The animals were placed in a screened room. Fine needle electrodes were inserted into the central and distal portions of the muscles. To stretch the muscle a load was hung on the lower jaw, or else the muscle was partially separated and a load attached to the distal end. The distal end of the m. masseter was separated together with a small portion of bone. During the experiments the cats were kept under light ether anesthesia.

EXPERIMENTAL RESULTS

A. Tonic innervation at rest. These experiments established the presence of a tonic innervation of the jaw muscles in kittens 2-3 hours after birth.

The greatest electrical activity in the m. masseter occurred while feeding on milk. The amplitude of the potentials varied from 20 to 35 microvolts, and the discharge frequency was 120-130 per second. Later the amplitude of these potentials was reduced. Thus, while on the 13-14th day it had a value of 14-16 microvolts, at the end of the month it usually did not exceed 10 microvolts. The discharge rate was also reduced to 100-110 per second.

B. Reflex response to stretching the jaw muscles. Proprioceptor stretch reflexes in kittens are of the same general type as those in adult animals, but there is some variation with age, and in kittens the reflexes are not well-maintained and are rapidly inhibited. In kittens there is a widespread irradiation of proprioceptor excitation in the central nervous system. As in adult cats, the effect depends to a great extent on the amount the muscle is stretched.

The following are the experimental results. In kittens aged 0 to 5 days the m. masseter was stretched by a load of 10-20 g applied to the jaw, and this caused an increase of 10-15 microvolts in the amplitude of

the oscillations above the resting value, and a considerable increase in frequency. Further increase in the load caused a clonus of the lower jaw, and if the load did not exceed 50 g this was maintained as long as the tension was applied. If the load was greater (up to 100 g) clonus occurred only during the first few seconds, and then either ceased altogether or else appeared again after a short interval. Further increase of the load up to 110-200 g caused 2-3 clonic movements of the lower jaw which was followed by an almost complete relaxation of the muscle. The clonus took the form of rapid incomplete jaw closures. The electromyograph trace showed separate high amplitude impulses alternating with groups of low amplitude potentials (Fig. 1).

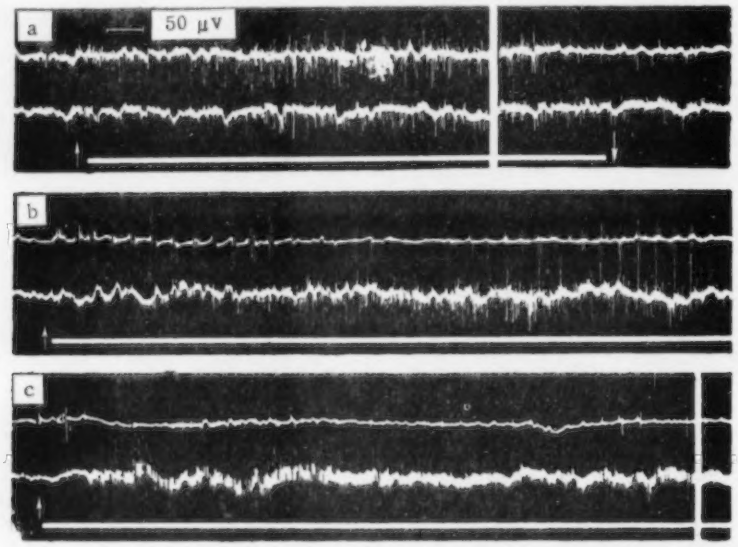

Fig. 1. Electrical changes in tensed and untensed muscles. Upper trace: Tensed muscle (m. masseter), lower trace – m. digastricus.

Load applied to jaw: a) 20 g, initial amplitude of oscillation 30-40 microvolts; with tension of 40 g – 50 microvolts, individual impulses 70 microvolts; b) 50 g, c) 100g: b) and c) clonic jaw movements in response to tension.

Experiment on cat 2 hours after birth. In all traces ↑ indicates application of load, ↓ – time of removal of load.

At the age of 5-15 days a load of 20-50 g, and from the 15-30th day a load of 50-100 g usually gave a uniform increase in the amplitude of the potentials and an increase in the frequency for the whole period for which the tension was applied. Greater loads applied subsequently caused large volleys of impulses which lasted from a fraction of a second to several seconds depending on the extension of the muscle. The large potentials alternated with small ones which sometimes occurred only occasionally, and at other times had quite a high frequency. An excessive tension of 200-300 g caused an initial increase in the discharge followed by an inhibitory effect (Fig. 2).

In the above experiments the tension was applied to the m. masseter by attaching weights to the lower jaw. With this arrangement, besides the receptors of the masseter muscles, the sensory endings of the whole group of muscles of mastication (m. temporalis and m. pterygoideus), as well as those of the skin round the mouth might be stimulated. Control experiments showed that the principal effect in the contraction of the masseter muscle was mediated by stimulation of proprioceptors in this muscle itself. This was shown in experiments in which the masseter muscle was stretched via the jaw after denervation of all the other jaw muscles on both sides, and also when the skin and subcutaneous tissue round the mouth were anesthetized. In experiments

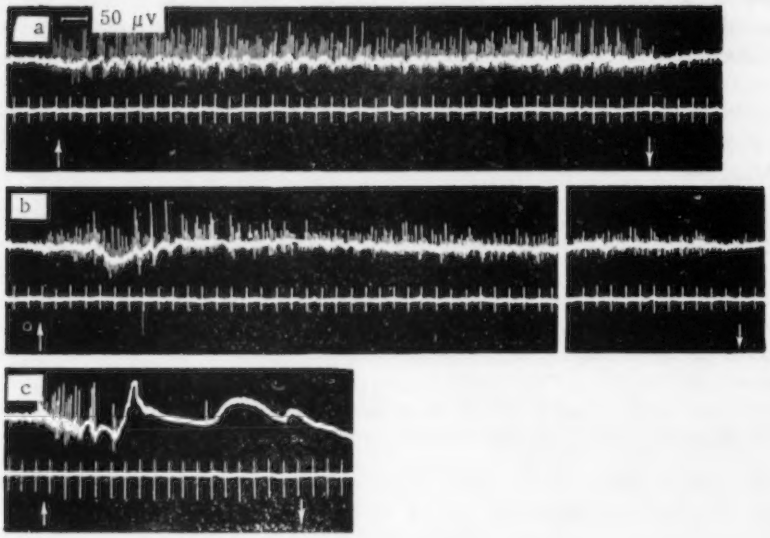

Fig. 2. Effect of tension on potentials in m. masseter in 5 day kitten. Upper trace – electrical discharges in the muscle; lower trace – time intervals of 0.1 seconds. Tension of muscle through load on jaw: a) 50 g, b) 150 g, c) 200 g.

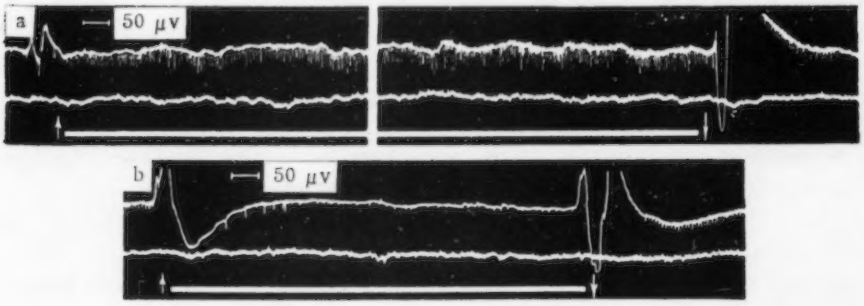

Fig. 3. Electrical changes on stretching the muscle.
Upper trace – tension applied to detached m. masseter; lower trace – muscle of the opposite side. Extension of muscle by load: a) 20 g, b) 100 g.

Experiment on cat aged 6 days. Humps in the line due to movement of electrodes at attachment and removal of load.

in which the masseter muscle was partially separated from the upper jaw and was indirectly stretched by tension applied to its separated distal end, it was shown that reflexes occurred less constantly. Also they differed from the corresponding reflexes of muscles with intact connections by being poorly maintained and rapidly extinguished. It is probable that this is due not only to the trauma associated with the separation of the muscles from the bones, but also the exclusion of other reinforcing "innate" reflexes which occur in stretching the jaw muscles through movements of the jaw.

For comparison we give the electromyograms from experiments on kittens all approximately 5-6 days old. While a tension of the undetached muscle from loads up to 150 g (Fig. 2) was able to produce a well-maintained reflex, inhibition developed very much more quickly on stretching individual muscles (Fig. 3). In stretching a detached muscle with a load of 20 g, the amplitude of the electrical potentials increased from 25-30 to 30-40 microvolts. A 50 g load caused no diminution of the action potentials, but caused a decrease in discharge rate. A load of 100 g not only reduced the rate but also reduced the amplitude of the potentials to less than one half of their original value.

C. Irradiation of proprioceptor excitation. Excitation occurring in kittens on stretching the jaw muscles spreads very readily to neighboring skeletal muscles and to the respiratory center. The younger the kitten, the smaller are the loads required to produce this irradiation. In a kitten 2 hours after birth a tension of 20 g applied to the masseter muscle caused an increase in the tonic muscle tension of the unstretched digastric. A greater tension on the masseter caused an increase in respiration which was visible to the naked eye. The increased excitation of the respiratory center led in turn to irradiation on to the nuclei of the principal and the accessory muscles of mastication. This caused an increase of the inhibitory processes in the stretched muscle and an increase in the excitation of the centers of the unstretched muscles. This explains the increase in electrical activity in the digastric, and the simultaneous reduction in the masseter (see Fig. 1).

Thus the present investigation confirms that the jaw muscles are functional at birth. This is shown by the tonic excitation of the masseter muscle at rest, and by the stretch reflex occurring a few hours after birth.

It must be supposed that the development of proprioception in the masseter is one of the necessary conditions for the development of the complex coordinated act of sucking.

From these experiments we conclude that the masseter muscle in kittens is maintained in a state of tonic excitation from the first hours after birth. The maintained muscular tension is greater during the time of suckling than later on, and is associated with the dominant influence of nutritional movements.

The jaw muscles in 2 hour old kittens show stretch reflexes. The proprioceptor stimulation involved in these reflexes gives rise to some irradiation in the central nervous system.

SUMMARY

The tonus and proprioceptive reflexes of extension of muscles of mastication were studied in experiments on kittens with the aid of the electrophysiological method. It was established that the muscles of mastication show electric activity from the very first hour after the birth of kittens. Broad irradiation and low stability are the specific features of the proprioceptive reflexes which were under investigation.

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REFLEX TONE IN THE TONGUE MUSCLES

(ELECTROPHYSIOLOGICAL INVESTIGATION)

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Tone in skeletal muscles is maintained by reflex action. One of its important features is that it is capable of maintaining organs in a definite position without noticeable signs of fatigue.

The reflex mechanisms of the maintenance of muscle tone in the limbs and trunk has been very fully studied. The tonic reflexes of the eye, ear and other muscles have also been described.

However, up till now no investigation has been made of the tongue muscles. The present work is devoted to an investigation of this problem.

EXPERIMENTAL METHODS

The muscle tone of the tongue was recorded with a myograph and an electromyograph. The latter recorded the muscle potentials of the tongue by means of an amplifier and a cathode ray oscillograph. The potentials were led off by needle electrodes. The experiments were carried out on cats.

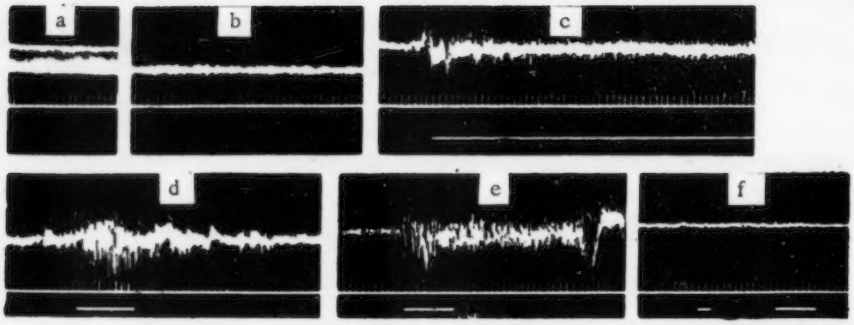
The proprioceptors of the tongue were stimulated by stretching the tongue with a load of 20, 50, 100 and 200 g. The duration of the stimulus was from 0.1 to 5 seconds. A pneumatic method was used to record the stimulus on the film.

EXPERIMENTAL RESULTS

Investigation of the tongue muscle potentials showed that after section of the sensory nerves supplying the mucous membrane of the mouth (lingual, glossopharyngeal and superior laryngeal), when only the hypoglossal nerves were preserved, a fairly regular discharge could be recorded from the tongue muscles during rest. They had an amplitude of 40-45 μv and a frequency of 30-35 c/s (see Fig. b). These potentials represented lingual tone at rest.

After recording the background noise due to spontaneous activity the tongue was stretched by hanging on to it weights of 20 or 50 g. After this, as a rule, the amplitude of the potentials increased to 60-80 μv and the frequency to 80 c/s or more (see Fig. c).

After this increase in tonic muscular activity of the tongue, increasing the load by a further 100 g immediately caused volleys of potential of amplitude 250 microvolts and frequency 100-150 c/s. These powerful volleys of bioelectric potentials were maintained for the whole of the time for which the load was applied, and even for some seconds after it had been removed (see Fig. d). The after discharge gradually died away, rapidly at first, and then more slowly. If the load was not removed, the discharge rate became considerably reduced after a certain time.



Electrical activity of the tongue muscles. Traces (from above downwards): action potentials of the tongue muscles, time intervals of 0.1 seconds, mark showing stimulation.
a) Calibration signals. Amplitude 50 microvolts. Amplification the same for all traces; b) electrical activity of the tongue at rest (without load); c) activity on stretching with load of 60 g; d) activity on stretching with load of 100 g; e) activity on stretching with load of 200 g; f) absence of electrical activity on stretching with load after section of hypoglossal nerves.

A further increase of the load to 200 g during well-shown tonic activity caused a still stronger effect – the amplitude of the potentials was 200-250 μv, and isolated potentials reached a value of 300 or more μv, while the frequency rose to 200 or more c/s (see Fig. e). There was a considerably longer after discharge which lasted up to 2-3 and sometimes 5 minutes.

Stretching the tongue with a load of 250-300 g at the moment of applying the load produced volleys with an amplitude of 200 microvolts, and a frequency of 70-80 c/s after which there was a marked reduction in activity. Isolated potentials were recorded. Thus increase of the load beyond a certain optimal value instead of causing an increase in the potentials, caused a suppression of the electrical activity and even a disappearance of the background potentials. If this overload was removed after 3-7 seconds the background bioelectric potentials appeared again.

Besides stretching the tongue rapidly and for short periods we also tried the effect of a very gradual extension. In this case the volleys of potentials described above were not observed.

Increase in electrical activity occurred not only in the case of longitudinal extension, but also with transverse stretching and in response to compression, (but only when this was short and sharp). In these cases the amplitude of the potentials was 50-60 μv the frequency – 70-80 c/s, and the potentials disappeared when the stimulus was removed.

In further experiments tension was applied to the tongue by hanging on weights of 50-100 g. After the appearance of the potentials described above, the two hypoglossal nerves were cut simultaneously. At the moment of the nerve section a stream of impulses appeared, but after 1-2 seconds this sharp burst of electrical activity ceased. After preliminary bilateral section of the hypoglossal nerves neither stretching nor compressing the tongue caused any potentials to appear.

In several experiments a bilateral novocaine block of the hypoglossal nerves, using 1% novocaine, was made. After 8-10 minutes from the time of the novocaine injection, there was a sharp reduction of the potentials associated with the normal tone of the tongue, and after 12-15 minutes the potentials ceased altogether, after which neither stretching nor compressing the tongue and the hypoglossal nerve caused any potentials to appear. There was a considerable lengthening of the tongue which hung out of the mouth. After washing out the novocaine for 20-30 or more minutes the previous potentials returned, but were weaker.

Thus the normal tone of the tongue muscles and the potentials associated with it are reflex in nature and closely associated with the lingual nerve centers. Proprioceptor impulses from the tongue muscles pass into the central nervous system, causing tonic excitation of the centers controlling the tongue muscles.

SUMMARY

The tonus of the tongue muscles was studied in animals. Experiments were performed on anesthetized cats. The tongue was stretched with weights ranging from 20 to 30 g. Reaction of the tongue to stretching was registered by a cathode ray oscillograph. It was established that the more the tongue is stretched, the greater its electric activity i. e. the more pronounced the proprioceptive reflex of stretching. Section of hypoglossal nerves destroys the tonus of the tongue muscles "at rest" , as well as proprioceptives reflexes to stretching.

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•• Russian Translation.

THE EFFECT OF ACETYLCHOLINE ON THE RESTING CURRENT OF A NERVE•

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Since acetylcholine formation occurs regularly during the process of excitation in many parts and in particular in myelinated nerve fibers, the question arises as to its possible role in the conduction of impulses along a nerve fiber, and so in turn to the possible electrogenic effects of acetylcholine, i.e. its effect on the electrical properties of the nerve.

According to M.A. Menshikova [11], acetylcholine causes a maintained depolarization of nerves bathed in Ringer's solution. This result does not agree with the findings of Lorente de No [13]. In his experiments, acetylcholine only caused a delay in anoxic depolarization, and a more rapid recovery after it. The polarizing effect of acetylcholine has been observed by E.B. Babsky, A.G. Mershchikov and F.D. Sheikhon [5] in experiments on the circumesophageal commissure of the Anodonta. However, Barnes and Beutner [12] maintain that acetylcholine has no effects on the resting current of a nerve immersed in saline (Ringer), but depolarizes the nerve placed in isotonic glucose. S.D. Kovtun [10] found that acetylcholine had no effect on the resting current. In view of this disagreement we investigated the action of acetylcholine on nerves immersed in Ringer solution and in a solution of glucose.

EXPERIMENTAL METHOD

The isolated sciatic nerve of the frog (Rana temporaria) was placed lengthwise in an ebonite bath in a special groove. At the bottom of the bath there were holes 1 mm in diameter through which contact with the nerve was made by nonpolarizable Du Bois-Reymond electrodes. The groove of the bath was divided by vaseline partitions into two or three sections. Each section was filled with a solution bathing the part of the nerve investigated. We used Ringer's solution, and isotonic and hypotonic (1.8%) solutions of glucose. The resting current was recorded using a mirror galvanometer (sensitivity 1 mm at 1 m = 0.53×10^{-9} A; R = 2065 ohms; R_{cr} = 10,835 ohms; T = 5.9 seconds). The potential difference between two portions of nerve was measured using a compensating arrangement. Acetylcholine was used in concentration of 10^{-3} and 10^{-4} (in concentrations of 10^{-5} it had no effect) and was placed on the nerve by two methods: it was either dissolved before the experiment in the solution, or it was added to it in drops during the experiment.

• The work was carried out under the direction of Professor E.B. Babsky in the Physiological Laboratory of the Moscow V.I. Lenin Pedagogical Institute.

EXPERIMENTAL RESULTS

I. Effects of Acetylcholine on the Resting Current of a Nerve Immersed in Ringer's Solution

Two of the sections of the bath in which the nerve lay were filled with Ringer's solution. The difference of potential between the two portions of the nerve was measured before and after adding acetylcholine. Acetylcholine in a concentration of 10^{-3} was added at first to one, and after a considerable time to the other portion of the nerve. The nerve was treated with acetylcholine either by replacing the Ringer's solution with a solution of acetylcholine in Ringer (10 experiments), or the acetylcholine solution was added in drops to the Ringer solution (10 experiments). Acetylcholine had no effect in any of the experiments.

II. Effect of Acetylcholine on the Resting Current of a Nerve Immersed in Glucose Solution

The proximal portion of the nerve was immersed in isotonic glucose, and the distal in an isotonic solution of KCL. Acetylcholine was added to the electropositive proximal portion. The change of potential difference was so rapid that we had to abandon introducing acetylcholine together with the solution. Therefore in most experiments of this group, and in subsequent experiments, acetylcholine was added in drops to the solution containing the nerve. In a concentration of 10^{-4} it caused a change of potential difference of 1-3 mv which reached a maximum value in 5-10 seconds and fell to zero after 10-15 minutes. In all 8 experiments of this group an increase in positivity was observed. In a concentration of 10^{-3} acetylcholine causes a more marked change of potential difference. In some of the experiments both parts of the nerve were placed in the solution and acetylcholine added in turn to each portion. Before the beginning of the experiment the nerve was kept for 60-90 minutes in the glucose solution. Sometimes a positive (Fig. 1) and sometimes a negative (Fig. 2) effect was produced and quite frequently the two effects could be observed on different parts of one and the same nerve (Fig. 3). Out of 100 experiments carried out to investigate this twofold action of acetylcholine, negative changes were found in about 10%, and positive changes in about 90% of the cases. The maximum positive change was 28 mv and the maximum negative − 21.7mv. On the average the change in potential difference was 8-10 mv. The change occurred immediately after adding the acetylcholine and reached a maximum value after 5-30 seconds or more; the return to the initial potential difference was 80-90% complete in approximately the same time; this initial potential difference was completely re-established after 5-10 minutes, or in some cases after 20-30 minutes. In most of the experiments the change in the potential difference ended by reaching approximately the initial value. However in certain cases the change proceeded further in the direction opposite to the initial one. In control experiments a drop of sodium chloride solution was added to the nerve immersed in the glucose solution. An average positive potential change of 3 mv was produced (16 observations).

III. Investigation of the Twofold Effects of Acetylcholine

a) Dependence of the effect of acetylcholine on the time of immersion of the nerve in the glucose solution.

The nerve was immersed in a glucose solution. The initial potential difference was determined. Immediately after this acetylcholine was added first to one portion, and then after a definite interval of time to the other. From 38 observations made during 21 experiments a definite relation was found between the absolute value and the sign of the effect of acetylcholine (whether positive or negative) and the time of immersion of the nerve in the glucose solution. In 20 out of these 38 observations a time of from 1 minute to 6 minutes elapsed between the immersion of the nerve in the glucose solution and the action of acetylcholine on it. A positive effect was found in 12 cases; the maximal positive effect was 22.5 mv, the minimum − 0.3 mv, the average − 6.2 mv; an electronegative effect was found in 8 cases, the maximum value was 15.5 mv, the minimum − 1.4 mv, and the average − 4.9 mv.

In the remaining 9 of the 38 observations, the time interval between immersing the nerve in the glucose solution and the action of the acetylcholine was 10-34 minutes; a positive effect was found in 8 cases, the

maximum being 17.5 mv, the minimum – 1.0 mv and the average 7.1 mv; a negative effect of 16.5 mv was found in one case.

In the remaining 9 cases, the time between the immersion of the nerve in glucose solution and the addition of acetylcholine was 70-195 minutes; the maximum positive effect was 34 mv, the minimum – 13.8 mv, and the average – 23.3 mv; no negative effect was found, the change being positive in every instance.

From these results we concluded that the longer the nerve remains in glucose, the greater is the positive potential developed, and the fewer are the cases where a negative effect is produced in response to acetylcholine. It seemed probable that the electronegative effect of acetylcholine is associated with the condition of the nerve in the early stages, and the electropositive with the later stages of immersion in glucose solution. Actually, in 13 of the 17 experiments in which the interval for the first portion of the nerve treated with acetylcholine was equal to 1 minute, and that for the second 2-6 minutes, a negative effect was found. In the case of a treatment with acetylcholine repeated after 30-40 minutes, a positive effect only was produced. In a careful study of the action of isotonic glucose on the resting current we found that a short electropositive phase lasting a few minutes preceded a steady electronegative phase; in these experiments one part of the nerve was placed in Ringer and the other in glucose solution. However in subsequent experiments it was found that the sign of the acetylcholine effect did not depend on whether or not a positive effect had occurred in the glucose solution.

In several experiments the nerve was placed in a hypotonic glucose solution. In these cases too, a double acetylcholine effect was found, i.e. both positive and negative potentials were developed.

b) Action of acetylcholine on the dead nerve

Experiments in which it was shown that acetylcholine exerts only an electropositive effect on nerves kept for a long time in glucose solution, suggested that in the glucose solution considerable changes in the functional condition of the nerve take place, and that these are due to the passage of ions out of the nerve. The question then arose as to how a nerve which had suffered irreversible changes, i.e. a nerve which had been killed, would react to acetylcholine. To this end a nerve was placed for 30 seconds in boiling Ringer, and then kept in glucose solution for several minutes. Acetylcholine was placed in succession on both portions of the nerve. No negative effect was found in any of this group of experiments. At the same time the effect of acetylcholine was approximately twice as great (in several experiments about 50 mv, on average – 20 mv), and the positive effect developed and ended more rapidly than in the living nerve. Within the limits of accuracy possible with such large fluctuations, the potential difference returned to its original value after the action of acetylcholine.

DISCUSSION

We were not able to confirm the results of M.A. Menshikova on the depolarizing action of acetylcholine, and concluded that acetylcholine does not affect the resting current of a nerve immersed in Ringer. The same conclusion was reached previously by Lorente de No and Barnes and Beutner. According to Netter [15], the electrogenic effect of substances is greatly reduced by immersing the nerve in saline. In studying the action of acetylcholine on a nerve immersed in a salt-free medium, Barnes and Beutner found a depolarizing effect. In similar experiments we found that the effect could be either negative or positive.

In many experiments designed to reveal the part played by acetylcholine in nervous activity, the double action of acetylcholine occurs. It is well shown in the action of acetylcholine on the reflex function of the nerve centers [3,4,7,8], and on their electrical activity. We found this same double effect of acetylcholine in the present investigation. From our results it appears that the recorded changes in the demarcation potential, due to the action on the nerve of acetylcholine, can at any one moment be considered to be made up of the sum of two components: the electropositive and the electronegative. One or the other of these two components may preponderate according to the time at which the acetylcholine acts on the nerve immersed in the glucose solution. It is abundantly clear that the nerve thus immersed undergoes changes in its functional condition which show up in a study of the demarcation potentials as a predominance of the positive acetylcholine effect. This conclusion is indirectly confirmed by the results of the experiments on the action of acetylcholine on the killed

nerve, in which there was no electronegative effect, and the positive effect was twice as great. We conclude that the electronegative effect of acetylcholine is characteristic of the living nerve only, and that it has a functional significance.

Our results on the double action of acetylcholine not only disagree with those of Barnes and Beutner, but also are opposed to the theory of Nachmansohn [14] on the exclusively depolarizing role of acetylcholine. Here we reach the same conclusion which was stated previously by E.B. Babsky [6] and A.G. Ginetsinsky and Z.I. Barbashova [9].

Further investigation is required of the problem of the nature of the changes which occur in the nerve when immersed in isotonic glucose, and which determine the sign of the acetylcholine effect.

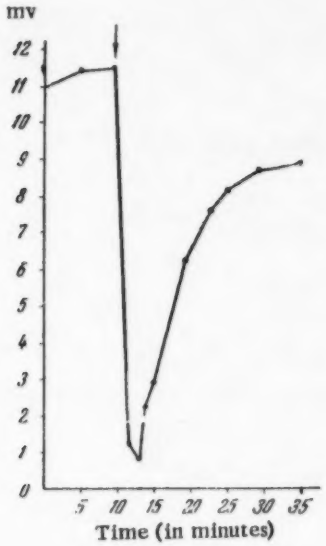

Fig. 1. Positive electrical change caused by acetylcholine (|).

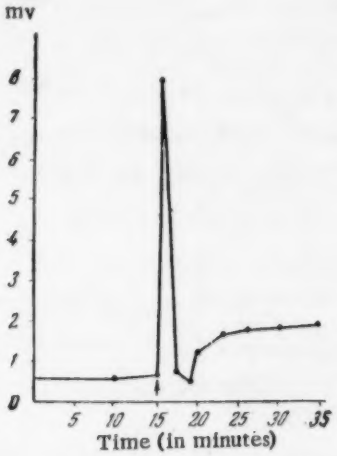

Fig. 2. Negative electrical change caused by acetylcholine (|).

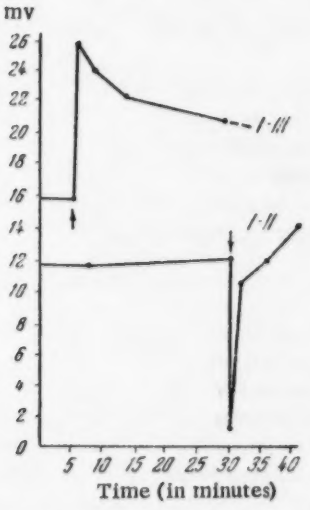

Fig. 3. Electropositive (leads I and II) and electronegative (leads I and III) effects caused by acetylcholine on different parts of the same nerve.

SUMMARY

It was established in experiments on Rana temporaria that acetylcholine has no effect on the demarcational potentials of the nerve, immersed in Ringer solution. Acetylcholine in 10^{-4}, 10^{-3} concentrations acts on the nerve immersed in glucose solution and causes the change of demarcational potentials. Development of either electronegativity or electropositivity takes place in these conditions. The effect of acetylcholine depends on the duration of immersion of the nerve in the glucose solution. The longer it is soaked in this solution –the more the absolute value of electropositivity and the less the number of cases with electronegative effect. Electronegative effect produced by acetylcholine is the inherent property of the live nerve.

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FURTHER DEVELOPMENTS OF A. A. UKHTOMSKY'S THEORY OF THE PARABIOTIC ORIGIN OF THE REFRACTORY PHASE*

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(Received September 3rd, 1955. Presented by Active Member of the Academy of Medical Sciences, USSR, D.N. Nasonov)

The present work constitutes the first experimental proof of the theory of A.A. Ukhtomsky [9] that the refractory phase of the nerve is a short lasting condition of parabiosis.

Many results have been published which suggest that the refractory phase is a manifestation of parabiosis. D.G. Kvasov and G.E. Zeberg [7] showed that the minimal time taken for cathode block, established by N.E. Vvedensky's method of minimal polarization, to disappear, corresponds to the duration of the absolute refractory phase.

D.S. Vorontsov [4] observed that during the relative refractory phase, the cathode of a strong inductive stimulus inhibits the nerve impulse, particularly when the nerve is treated with potassium. As a result of this, the cathode of a second stimulus increases the duration of the absolute refractory phase, while the anode reduces it. Similar effects of the action of the anode and cathode of a second stimulus on the nerve impulse at the very beginning of the relative refractory phase have been described by the Japanese authors Otani, Ono and Usio [12]. Consequently, in the relative refractory phase, the anode and cathode affect the development of a nerve impulse in a way which resembles their action on a nerve which is in a condition of parabiosis due to potassium, i.e. the cathode increases and the anode decreases it (M.I. Vinogradov) [3].

Thus it may be supposed that during the relative refractory phase the nerve passes into a short period of parabiosis. If this is so, at the very onset it ought to be possible to observe the typical stages of parabiosis, namely the paradoxical and equalizing phases. Indirect experimental evidence for this idea is given by A.N. Kabanov [6]. However there is no direct proof that a parabiotic stage occurs at the beginning of the relative refractory phase. Professor N.V. Golikov has told us that in 1930, A.A. Ukhtomsky entrusted him and his student P. Shmidt to investigate this problem. However technical reasons prevented him from doing so.

EXPERIMENTAL METHOD

Monophasic action potentials from the isolated sciatic nerve of the frog were recorded on a four channel cathode ray oscillograph [5].

Three of the beams of the cathode ray oscillograph were used. Two-millisecond time markings from a 500 c/s tuning fork generator were recorded on the upper trace, stimuli from a special generator on the middle

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*Presented June 25, 1955 at the A. A. Ukhtomsky Physiological Institute, Leningrad State University, at the meeting in honor of the 80th anniversary of the birth of A. A. Ukhtomsky.

trace, and the nerve action potential on the bottom trace. The generator allowed 2 stimuli of triangular or square wave form of pre-established length to be delivered. A stimulus length of about 0.5 milliseconds was used. The interval between these two stimuli could be varied from 0 to 100 milliseconds. The amplitude of the stimuli could also be changed over a considerable range. The setup was arranged to give a steady picture, so that both photography and visual observation were possible. The steady picture on the screen was obtained by not using isolated pairs of stimuli, but a series of them at a frequency of 100 per second. Besides being able to stimulate the nerve with the series of double stimuli, a single pair of stimuli could be applied once, as in the usual pendulum arrangement. Both methods gave the same result. The traces shown below were recorded using the steady repetitive picture.

EXPERIMENTAL RESULTS

Figure 1 shows five oscillograms recorded in succession from the same nerve. Oscillogram a shows the action potential developed in response to the first stimulus. Oscillogram b shows two action potentials: the first large potential is due to the first stimulus, and the second small one to the second stimulus, delivered 2 milliseconds after the first one, i.e. at the very beginning of the relative refractory phase. Oscillogram c is taken with the same interval between the first and the second stimuli but with a much greater amplitude of the second stimulus, with the result that the second action potential has almost disappeared. If the amplitude of the second stimulus is now made smaller, as is shown in oscillogram d , then the second action potential again appears. Oscillogram e shows that if a single stimulus is given again,the picture obtained is precisely the same as oscillogram a , which shows that the condition of the nerve has not changed. Comparison of b, c and d shows that the paradoxical phase of parabiosis is occurring.

When the interval between the first and second stimuli is increased the equalizing stage of parabiosis occurs. Figure 2 shows five oscillograms taken from the same nerve used in Fig. 1. In order to be able to show the greatly increased amplitude of the second stimulus, a reduced amplification was used for the stimulus trace. The first stimulus here, as in Fig. 1, is supramaximal. This time the second stimulus was applied approximately 4 milliseconds after the first, i.e. somewhat longer after the beginning of the relative refractory phase than in the previous experiment.

While maintaining the interval between the stimuli constant, the amplitude of the second stimulus was increased (Fig. 2, oscillograms b,c ,d and e). The action potential developed in response to the second stimulus, increases at first (Fig. 2,b), and then remains at a steady value (Fig. 2, c,d and e).

The results of the experiment shown in Fig. 2, b,c,d, and e are to be interpreted as showing the equalizing stage of parabiosis.

DISCUSSION

What objections are there against paradoxical changes in the action potential occurring in response to variation in stimulus strength, as shown in the oscillograms? On increasing the amplitude of the second stimulus, the action potential evoked by it at the very beginning of the relative refractory phase almost completely disappears. It follows that the activity of all the fibers of the mixed nerve trunk has been suppressed.

Are not other characteristics of the action potential also changed with increase of stimulus strength? P.O. Makarov [8] has shown that the length of the refractory phase may be changed, for instance by alteration of the shape or duration of the stimulus. However our results contradict this idea. They show that the shape, rate of rise and duration of the stimuli remained unchanged for the whole of the experiment; only the strength of the second stimulus is changed. This allows us to attribute changes in the size of the action potential solely to change of stimulus strength.

It seems paradoxical to say that variations in the amplitude of an action potential which obeys the "all or none" law, represent the equalizing stage of a functional parabiosis which is itself derived from an action potential, or, more precisely, from the catelectrotonic portion of a spike.

Approaching the problem in a different way, it may be asked whether the equalizing stage of parabiosis does not itself constitute the means whereby the control is effected over the nerve impulse. This was the standpoint of L. M. Shereshevsky and N.A. Shoshina [11] and A.A. Ukhtomsky [9]. They considered that the alteration of the action potentials must be interpreted as representing the equalizing phase of parabiosis. The problem of the reason for the autoregulation of the nerve impulse was considered by N.E. Vvedensky [2]. Our experimental results showing the equalizing stage of parabiosis in the relative refractory phase go some way towards supplying the answer to the problem raised by N.E. Vvedensky, and support the view of L.M. Shereshevsky, N.A. Shoshina and A.A. Ukhtomsky.

It is not possible here to go into a detailed discussion about the mechanism of autoregulation of nerve impulses. We will merely refer to the work of M.S. Averbakh and D.N. Nasonov [1] which deals with this problem. They consider autoregulation of nerve impulses to be based on only one of the indices of the functional condition of the nerve, namely its excitability.

In view of the results we have described here, it must be supposed that in the mechanism of autoregulation, not only does the excitability of the nerve play an important part but so also does the parameter of physiological lability.

Thus we have shown experimentally, that during the relative refractory phase, the amplitude of the action potential of the nerve changes, according to the interval between the conditioning and the test stimulus, in accordance with the laws of the parabiotic process, so that the paradoxical and the equalizing stages of parabiosis are shown. The existence of these stages has also been demonstrated in the case of the motor nerve endings.

It appears likely that the equalizing stage of parabiosis plays an important part in the mechanism of the control of the nerve action potentials.

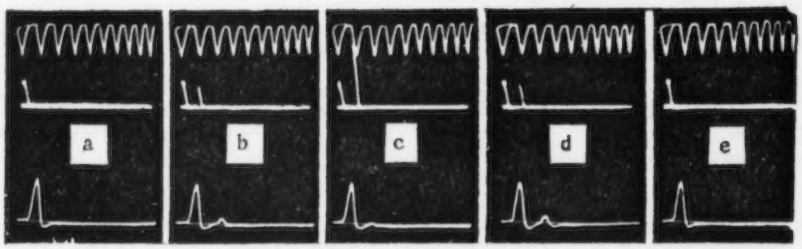

Fig. 1. Demonstration of the paradoxical stage of parabiosis at the very beginning of excitation of a nerve after the first maximal stimulus (at the beginning of the relative refractory phase).
Curves from above downwards: upper traces – time markings 500 c/s, middle trace – stimulus; lower trace – nerve action potential.
Oscillogram section a,b,c,d and e explained in text.

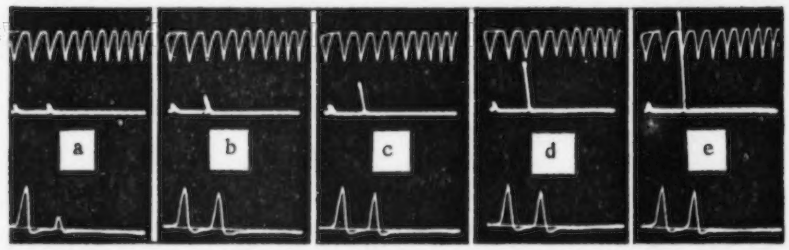

Fig. 2. Demonstration of the equalizing stage of parabiosis during recovery of excitability of a nerve after an initial maximal stimulus (in the relative refractory phase).
Key: same as for Fig. 1.

SUMMARY

In 1927 A. A. Ukhtomsky suggested that refractory phase of the nerve is the condition of short parabiosis. It is probable that during the relative refractory phase the nerve gradually comes out of parabiosis. If this is the case – then at the very beginning of it the typical stages of parabiosis should be found, i.e. the paradoxical and the equalizing. The author's experiment demonstrated that this actually takes place. It was experimentally established that during the relative refractory phase the amplitude of the potential of the nerve action changes. This change depends on the interval between the experimental and testing stimuli with the regularity of the parabiotic process. Paradoxical and equalizing stages of parabiosis were revealed at the beginning of the relative refractory phase.

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• In Russian.

EXCRETION OF UREA FROM HIGH BLOOD CONCENTRATIONS

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Of all the precise methods of estimating renal function which are available in a modern clinic, the determination of the urea clearance is the simplest, and therefore, of the greatest practical importance.

In diureses exceeding 2 ml per minute per square meter of body surface, the urea clearance is on the average, 70% of the true filtration, and its value is an indication of renal filtration capacity.

Theoretically this test is based on results which show that the renal tubules are purely passive with respect to urea: it is only filtered in the glomeruli and neither secreted nor reabsorbed actively, and is partially reabsorbed only through simple diffusion as the filtrate passes through the nephron [3,4,5]. The above is the commonly held view concerning the excretion of urea in mammals.

However, certain authors have held a different view. Thus, E. A. Asratyan [1] found in experiments on dogs that with high concentrations of urea in the blood, its clearance in certain cases might be almost twice as great as the total filtration. From this he concludes that there is an additional mechanism for the excretion of urea.

Recently K.M. Shteingardt [2] reached the same conclusion, after having repeated Asrayan's experiments and obtained results agreeing with his in every detail. The conclusions of these authors, which contradict the generally accepted view of the mechanism of the excretion of urea, have been often quoted in Soviet literature, and may cause doubt about the soundness of the method of determining the urea clearance as a means of estimating renal function.

With this in view, we carried out the work reported here.

EXPERIMENTAL METHOD

The experiments were carried out on dogs weighing 8-12 kg with the normal opening of the ureters brought out on to the skin of the belly. For the whole of the period under observation the animals were maintained on their usual liquid and solid diet.

The investigation consisted of a simultaneous determination of the filtration rate (inulin clearance) and urea clearance both with a raised blood urea, and under conditions of rapid diuresis, induced by other osmotically active substances. The animal was given a continuous intravenous injection of a solution containing 2% inulin and 2-10% urea at a rate of 3-5 ml/minute, or 2% inulin with 20-45% glucose solution. Urine and blood samples were taken after the injection had been maintained for 30-40 minutes, when steady concentrations of the substances in the blood had been reached.

Inulin in the urine and plasma was determined using a colorimetric resorption method, and urea – by the urease method as modified by Conway. From these results the filtration rates and urea clearances were calculated. The results were referred to $1 m^2$ of body surface, as this allowed comparison of results between dogs of different size and weight.

EXPERIMENTAL RESULTS

The results of 33 experiments on 4 dogs have been divided into three groups according to the blood urea concentrations which occurred with the loads used (Table 1).

TABLE 1

Average Values and Limits of Variation of the Ratio of Urea Clearance (C_u) to the Inulin Clearance (C_{in})

Number of experiments in the group	Limits of the observed concentrations of urea in the plasma (mg/ml)	Limits of variation of the ratio urea clearance (C_u) to inulin clearance (C_{in})	Average value of ratio C_u/C_{in}
9	from 0.11 to 0.30	from 0.57 to 0.91	0.72
11	" 0.31 " 0.50	" 0.60 " 0.97	0.77
18	" 0.64 " 2.20	" 0.72 " 0.98	0.85

As can be seen from Table 1, the average value of the ratio increases with increase in the concentration of blood urea. However, even with high concentrations up to 220 mg%, i.e. 10 times higher than normal, the ratio C_u/C_{in} remains less than 1, which shows that no active excretion occurs. At the same time with normal and moderately raised blood ureas this ratio varies considerably, sometimes reaching values of 0.91-0.97. Evidently there is no direct relation between the blood urea and the ratio of the two clearances. The increase in the average value of the ratio with increasing values of blood urea is probably not due to any specific effect of the urea on the tubules, but to the diuretic effect it produces. When diuresis is rapid, and when the rate of flow of the primary urine along the canals increases, conditions are less favorable for the reabsorption of urea, so that its clearance rises and approximates the true filtration rate.

It is known that over very wide limits of diuresis rates the filtration rate in dogs remains constant; however, the rate of flow of fluid along the canals, which clearly will be higher the greater the rate of urine excretion, is entirely determined by the rate of reabsorption of water. A convenient expression for the reabsorption process is the value of the ratio of the inulin content of the urine (u) to that of the plasma (p). The unreabsorbed inulin increases in concentration in proportion to the amount of water reabsorbed, and the ratio u/p increases. We would expect that the ratio C_u/C_{in} will depend on the ratio u/p.

In order to demonstrate this relationship in general, we altered the amount of water reabsorbed by injecting not only urea but also another osmotic diuretic which changes the value of u/p, namely glucose. The results of the experiment are shown graphically in the figure. The results of the experiments with intravenous injection of urea are shown by the open circles, while the closed circles show the results for glucose. It can be seen that by altering the concentration index of inulin from 40 to 15, the value of C_u/C_{in} varies from 0.8 to 0.6; however, with a concentration index less than 15, the value of C_u/C_{in} shows a steady increase, with a value of approximately 1.0 at a u/p ratio of approximately 4.0.

The four points lying above the dotted line must be noted. In these experiments the ratio C_u/C_{in} exceeds 1.0, and this can only occur if urea is actively excreted.

Table 2 shows the figures corresponding to these four points.

TABLE 2

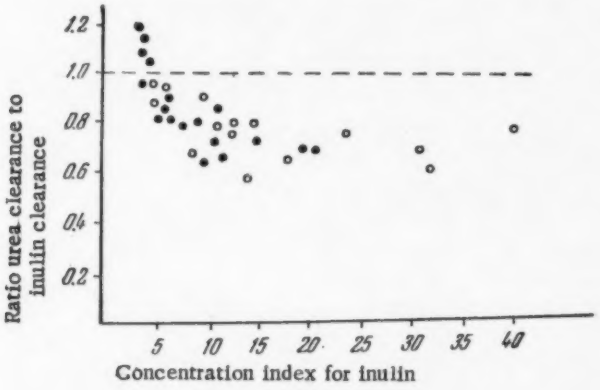
Excretion of Urea in Osmotic Diuresis Caused by Injecting Glucose

Concentration of urea in plasma (mg/ml)	Concentration index of inulin (u/p)	Diuresis in ml per m^2 per minute	Ratio of urea clearance to inulin clearance
0.35	4.5	22.0	1.05
0.35	4.7	23.8	1.08
0.20	3.9	24.7	1.16
0.32	4.0	20.4	1.20

Are we to conclude from these four experiments that under certain known conditions urea is actively excreted as E.A. Asratyan and K.M. Shteingardt suppose?

First of all it is to be observed that these exceptional values are not associated with a high blood urea, but were obtained in experiments with injection of glucose. In these experiments the diuresis was particularly high, and the u/p ratio for inulin particularly low; under these conditions the passive reabsorption of urine approximates zero, and the urea clearance approximates the filtration rate. We consider that under these conditions the true value of C_u/C_{in} is equal to unity. Values exceeding this amount in two experiments by 5-8%, and in two by 16-20%, are evidently due to an error, the most probable being failure to stabilize the ratios in the plasma during the collection of urine. In each instance the amount by which the ratio C_u/C_{in} differs from unity is in no way comparable with the differences found by E.A. Asratyan and K.M. Shteingardt which were of the order of 200%.

Their results are probably due to the fact that they measured filtration rate in terms of endogenous creatinin, which in dogs gives values which are too low, while for urea measurements they used the nonspecific methods of Foss and Borodin which are not capable of yielding sufficiently accurate quantitative results. Our results support the findings of those who hold that urea is excreted purely by filtration. Excretion is not an active process either in normal or in very high concentrations in the plasma.



Relation between concentration index for inulin and the ratio of urea clearance to inulin clearance. Open circles show results of experiments with injection of urea, dots – results of experiments with injection of glucose.

SUMMARY

The value of filtration by inulin method (C_{in}) and urea clearance (C_u) in its increased concentration in the blood were determined simultaneously. It was established that C_u/C_{in} ratio increases with reduction of concentration index of inulin caused by the loading of urea. The C_u/C_{in} ratio almost equals 1, but is never over it. Concentration of urea in the blood does not stimulate its excretion by the tubular epithelium.

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PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

INVESTIGATION OF THE MECHANISM IN THE PHYSIOLOGICAL ACTION IN CLOSED, ARTIFICIAL PNEUMOTHORAX

COMMUNICATION I. THE EFFECT OF ARTIFICIAL PNEUMOTHORAX UPON THE ACTION POTENTIALS OF THE VAGUS AND PHRENIC NERVES

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The widely-held opinion as to the mechanism of the respiratory act, the nature of the intrapleural pressure, and the mechanism of lung collapse following artificial pneumothorax has its basis mainly on purely physical concepts of lung biomechanics not associated with the physiological activities of the organism. In line with this concept, the therapeutic goal of artificial pneumothorax has been to put the lung at rest by means of filling the pleural space with air and thus either completely or partially collapsing the lung and so excluding its function of respiration. The logical explanation of the physiological and therapeutic mechanisms involved in artificial pneumothorax was the assumption that it was the rest which produced the desired result.

Contemporary physiological and clinical data which have been gathered [1,3,4,5,6,7,8,11,15] cannot be fitted into the old concepts of the biomechanics of the lungs during artificial pneumothorax, and demand an experimental study of this problem and, in part, a clarification of the role played by the nervous system in the mechanism of the action of artificial pneumothorax.

The present investigation was undertaken with the goal of studying the interoceptive reflexes of the respiratory apparatus as the organism becomes adapted to pneumothorax. In this communication are presented the experimental findings on the effect exerted by artificial pneumothorax upon the afferent impulses in the vagus nerve and the efferent impulses going down the phrenic nerve.

It is well known that electrophysiological studies have markedly widened and deepened our understanding of the mechanisms regulating respiration and have demonstrated the great effect upon the regulation of breathing exerted by afferent nervous impulses arising within the respiratory system itself from various mechanical and chemical stimuli.

Many investigators [2,9,10,12,13,14,16,17,18] have established the electrophysiological characteristics of the afferent impulses going up the vagus nerve and the efferent impulses travelling down the phrenic nerve during normal respiration and have proven their importance in the coordination of the respiratory act.

EXPERIMENTAL METHODS

These experiments were performed on rabbits and cats which were narcotized by means of a 15-20% solution of urethane injected into the femoral vein, the dose being 0.5-1 g per kg of animal weight.

The neck of the animal was opened and the vagus and phrenic nerves dissected clear of their sheaths.

As the vagus nerve within the cervical region has numerous afferent fibers conducting sensory impulses from the lungs, respiratory and digestive tracts, the heart, the arch of the aorta and the large veins, in our experiments we separated the main stem of the nerve into individual small bundles. Those bundles which were seen clearly to carry respiratory impulses were used for the testing of their electric potentials. Then the bundle was severed and the potentials were tested from the peripheral end of the cut. With the phrenic nerve, the potentials were tested from the central (upper) end of the cut.

The action potentials of the indicated nerves were obtained with the aid of platinum electrodes with a space of 3-5 mm between the poles. The impulses from the nerves were recorded by a double-ray cathode oscillograph produced in the experimental equipment factory of the Acad. Med. Sci. USSR. The frequencies of the amplifier were from 10 to 1500 cycles. During the experiments the animals were kept in a shielded chamber.

During the course of each experiment the potentials of the nerves during normal respiration before the induction of the pneumothorax, at the moment of producing the pneumothorax and at various times after that were registered in succession. Air was introduced into the pleural space in amounts of 25-50 cc, a regular pneumothorax machine being used.

EXPERIMENTAL RESULTS

As already stated in the literature [9,10,12,13,19], during normal respiration we observed regular oscillating streams in the vagus and phrenic nerves associated with the phases of the respiratory movements of the animal (Fig. 1, a). During inspiration, there was observed in the vagus an increase in the frequencies of the impulses and a rise in their amplitude, attaining a maximum at the height of inspiration. In the expiratory phase, the afferent impulses in the vagus nerve diminish sharply.

The volleys of efferent impulses in the phrenic nerve also have a characteristic pattern. Before the onset of inspiration and in the phase of its development the frequencies of the impulses and their amplitude increases to a given maximum, after which the impulses weaken sharply, disappearing entirely during expiration. This sharp decrease in the efferent impulses of the of the phrenic nerve with consequent total disappearance coincides in time with the moment of maximal development of the afferent impulses in the vagus nerve.

Thus, during normal animal respiration there is a very definite relationship between the afferent impulses of the vagus nerve and the efferent impulses of the phrenic nerve. The flow of interoceptive impulses from the respiratory apparatus to the respiratory center along the fibers of the vagus nerve is one of the factors assuring the regular alteration of the respiratory cycle.

When a closed artificial pneumothorax was induced in the animal, we observed material alterations in the afferent impulses of the vagus nerve and the efferent pulsations of the phrenic nerve. As can be seen from the oscillogram of Fig. 1,b,c, in the cat at the moment of inducing the pneumothorax there is registered in the vagus nerve a decrease of the afferent impulses in the inspiratory phase, while in the expiratory phase complementary impulses appear. At the same time, the efferent impulses in the phrenic nerve increase sharply. After some time, following the induction of the pneumothorax, there is observed a gradual restoration of normal relationships between the impulses in the vagus and phrenic nerves although the levels of the impulses differ from those seen before the application of the pneumothorax (Fig. 1,d,e).

The alterations in the action potentials just described that take place in the vagus and phrenic nerves of cats subjected to pneumothorax occur not only on the operated side but also on the opposite side, this being caused in these animals by an intercommunication between the two pleural cavities, so that a unilateral pneumothorax becomes bilateral.

The character and intensity of the action potential alterations in the vagus and phrenic nerves observed after the induction of pneumothorax depend on the amount of air being introduced into the pleural cavity and the speed of the operation.

When the air is introduced quickly into the pleural cavity, there frequently are seen instances of stoppage of breathing at the beginning of expiration, this being accompanied by continuous flow of impulses along the

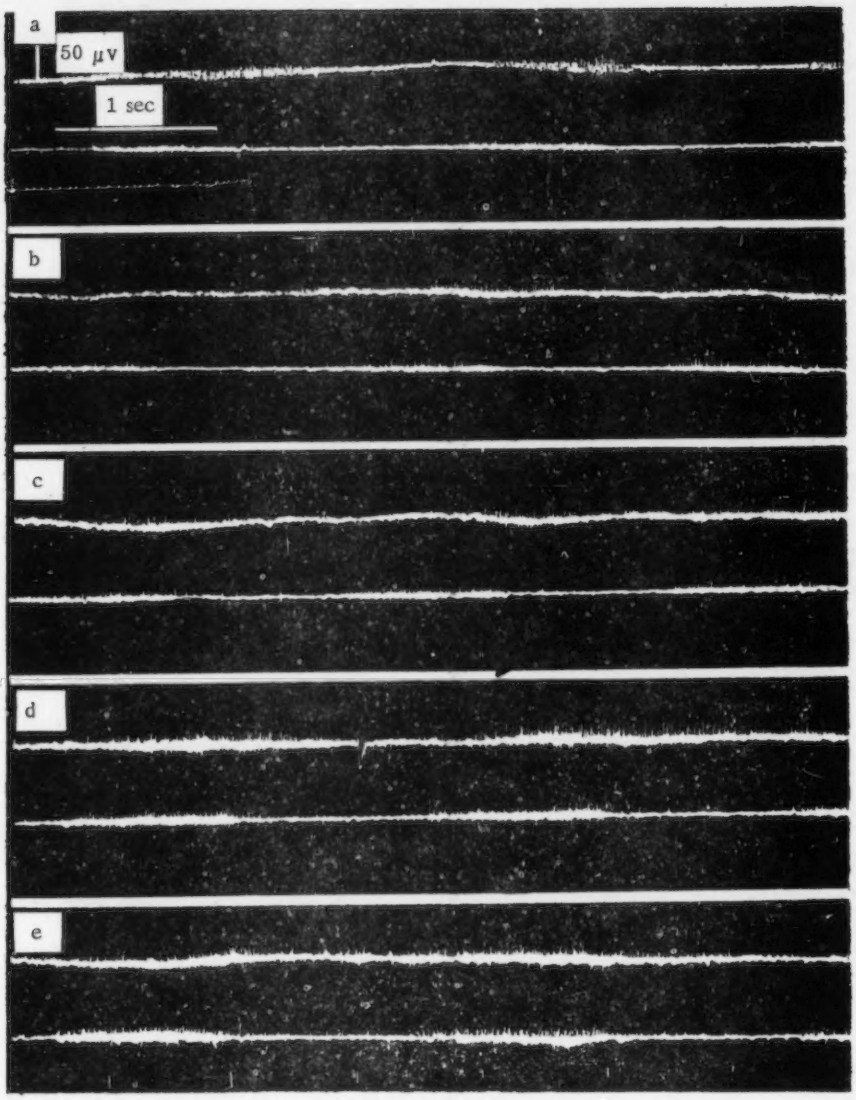

Fig. 1. Alterations in the afferent impulses of the vagus nerve and efferent impulses of the phrenic nerve upon induction of closed pneumothorax.
Significance of curves (from above): action potentials of left vagus nerve; action potentials of right phrenic nerve. Cat 1.9 kg. Experiment done October 30, 1954.
a) action potentials of vagus and phrenic nerves during normal respiration;
b) at moment of induction of pneumothorax by means of injection of 50 cc of air into the left pleural cavity;
c) immediately after the pneumothorax;
d) after one minute;
e) five minutes after the pneumothorax.

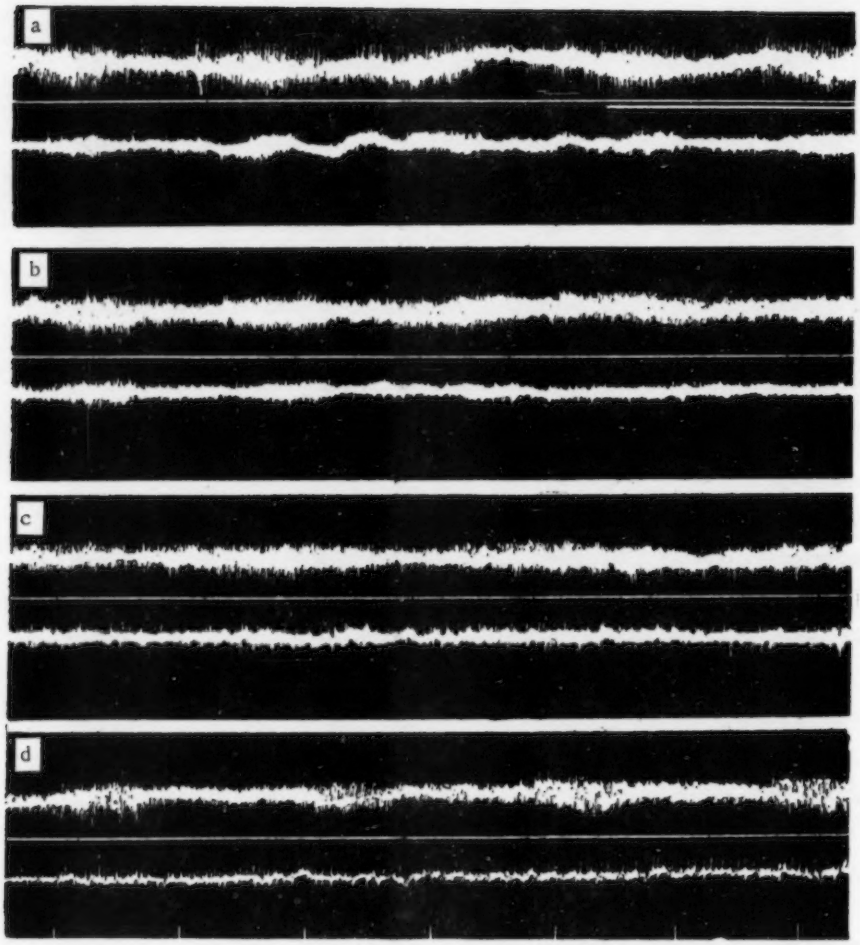

Fig. 2. Alterations of the afferent impulses of the vagus nerve at the induction of closed pneumothorax.
Rabbit weighing 2.5 kg. Experiment of January 7, 1955. Significance of tracings (from above): action potentials of left vagus nerve; action potentials of right vagus nerve.
a) initial back-ground; b) moment of inducing right sided pneumothorax $\frac{-6}{-4}$ 30 $\frac{-4}{+1}$; c) 30 seconds after the induction; d) five minutes after the pneumothorax.

phrenic nerve and a sharp weakening of the flow along the vagus nerve. The fact, recorded by us of the appearance of additional impulses in the afferent sensory fibers of the vagus nerve in the expiratory phase after the induction of pneumothorax, is in agreement with the studies by G. Knowlton and M. Larrabee [13] and by I. Widdicombe [17] as to the presence within the lung tissues of two forms of mechanoreceptors responding to stretching and compression of the lungs. Apparently, induction of pneumothorax affects the receptors reacting to lung compression, this being in the nature of a defense reaction to the collapse of the lung.

Analysis of the oscillograms indicates that, immediately after the induction of the pneumothorax and in the period of readjustiment to it, there is observed some increase of inspiration as compared to expiration when observed against the base level, this apparently being an aid to better gaseous exchange within the lungs.

Very characteristic is the circumstance that, following the introduction of 25-50 cc of air into the pleural cavity by inducing the pneumothorax, the level of the vagal afferent impulses is restored after a very short time to being as high or even higher than before the production of the pneumothorax. This demonstrates that the lung performs considerable expansion even during a hypotensive pneumothorax. This is further proven by the fact that in the presence of the pneumothorax the intrapleural pressure tends to return to the basic pattern characteristic for that animal [1,3], as well as compensatory expansion of the chest we have described previously [3], this occurring as a result of interoceptive reflexes from the pleura and lungs affecting the chest respiratory musculature.

We obtained interesting data in experiments upon rabbits. As is well known, the pleural cavities in rabbits are hermetically sealed off from each other. For this reason, induction of an unilateral pneumothorax in this animal leads to different functional states of the right and left lungs. This can be judged in the data obtained in our experiments of registering simultaneously the action potentials of the right and left vagus nerves with induction of an unilateral pneumothorax (Fig. 2).

The oscillograms in Fig. 2,a,b and c indicate that induction of the pneumothorax caused a sharp depression of the afferent impulses in the vagus nerve on the pneumothorax side and a compensatory strengthening of the vagal impulses on the opposite side. The action potentials of the phrenic nerves in this same experimental situation showed a marked strengthening of the efferent impulses in both the left and right sides. As the animal became adjusted to the pneumothorax, the relationships between the afferent volleys in the vagus nerve and the efferent volleys in the phrenic nerve on both sides tended to attain normal levels.

In some instances, the induction in rabbits of a tension pneumothorax would result in the suppression on the pneumothorax side for some time in the vagus nerve of the usual impulses associated with respiration and there would appear qualitatively new impulses not related to the respiratory movements of the animal (see Fig. 2,d). The character of this impulse resembles the action potentials registered by Widdicombe [16,17] in vagal afferent fibers coming from the tracheobronchial tree.

Thus the findings of our experiments indicate that induction of artificial pneumothorax affects the reflex zones of the respiratory apparatus, producing marked alterations in the character of the afferent signalization and, consequently, changing the activity of the respiratory center and aiding in the activation of compensatory adjusting mechanisms which assure the continued performance of the respiratory apparatus on a new level.

SUMMARY

The reflex arc involved in normal respiration was studied in relation to alterations occurring during and after the induction of artificial pneumothorax. Anesthesized rabbits and cats were employed. Intravenous urethane was the anesthetic agent. The afferent impulses in the vagus nerve and the efferent impulses in the phrenic nerve were tested by means of the standard oscillograph.

The lung is not simply put to rest mechanically by the pneumothorax. A number of adaptive compensatory mechanisms come into play assuring the continued activity of the respiratory reflexes under the new conditions.

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HYALURONIDASE ACTIVITY OF THE URINE IN NECROTIC NEPHROSIS

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Various pathological conditions of the kidneys are accompanied by characteristic changes in the hyaluronidase activity of the urine [1]. Thus, during the acute stage of diffuse glomerular nephritis, the activity of the enzyme is considerably increased, and the transition to the chronic phase is marked by a change in the hyaluronidase-diuresis curve, while in lipoid and amyloid nephroses, the hyaluronidase activity is reduced or even absent.

In the present investigation we studied the changes in hyaluronidase activity in experimentally induced necrotic nephrosis.

EXPERIMENTAL METHOD

Necrotic nephrosis was induced in dogs using Bobei's method [2] with subcutaneous injections of a 1% solution of uranium nitrate (2 mg per kg).

Symptoms were manifested most intensely one week after the injections. The general condition of the animal deteriorated, and signs of uremia appeared. Kidney function was reduced (urea clearance was 2.48 ml/minute/m^2, ie. it fell to 5% of the normal figure).

The protein content of the urine rose to 6.6%, and in the sediment hyaline casts and amorphous epithelial debris were found.

EXPERIMENTAL RESULTS

The results of the urine tests for hyaluronidase activity are shown in Fig. 1.

In the dog poisoned with uranium nitrate, the hyaluronidase activity is somewhat higher than normal. With a small diuresis it has a value of 30-33 units instead of the normal 20, and when the excretion rate reaches 1 ml per minute, when normally the activity falls to zero, it remains at a level of 20 units. No hyaluronidase activity was detected in the blood plasma.

The dog was killed two weeks after the injection. A histological investigation of the kidney confirmed a diagnosis of necrotic nephrosis.

We were also able to follow the changes in the hyaluronidase activity of the urine in kidney damage of the necrotic nephrosis type occurring in a human patient as a result of poisoning with corrosive sublimate.

The young woman, aged 19, was admitted to the town clinic hospital on December 13th, 1954 with hematemesis, and complaining of severe headache and a burning sensation in the mouth and esophagus. On that day she had drunk 150 ml of a solution of corrosive sublimate, intending to commit suicide.

Investigations were started 9 days after taking the poison. By that time the patient's condition was very grave, and there were clearly marked signs of nephrosis, stomatitis, colitis and cystitis.

The concentration of urea in the blood rose to 444 mg %. The urine, of which 900 ml were voided daily, was turbid and had the high protein content of 2.64%; in the sediment there were a number of leukocytes, hyaline casts, and various epithelial cells. Renal function was considerably reduced – the urea clearance was only 1.5 ml/minute, i.e. 3.6% of normal, while the concentration index of the urea at a diuresis of 0.74 ml/minute had a value of 1.8.

A bilateral decapsulation of the kidneys was carried out as one of the measures adopted to combat the anuria. During the operation, a portion of the capsule together with some adherent renal tissue was taken for histological examination. There was a necrosis and desquamation of the eqithelium of the convoluted tubules with minimal changes in the glomeruli, shown by a hyperemia, thus indicating a servere nephrosis of the necrotic type (Fig. 2).

The hyaluronidase activity was determined with a viscometric method in each spontaneously voided sample of urine.

As can be seen from Fig. 3, the hyaluronidase activity of the urine was markedly increased. At a diuresis of 0.3-0.5 ml/minute/m^2 it reached a value of 33 units, falling to 12-17 units when diuresis increased, and maintaining this value subsequently. Thus in the patient with necrotic nephrosis the hyaluronidase activity was higher than in a healthy human subject.

No increase in the hyaluronidase activity in the blood plasma was found.

This very limited material does not allow us to reach any definite conclusion about the reasons for the change in the hyaluronidase activity of the urine in necrotic nephrosis. The effect would appear to be due to the destruction of renal tissue setting free the enzyme contained in the cells.

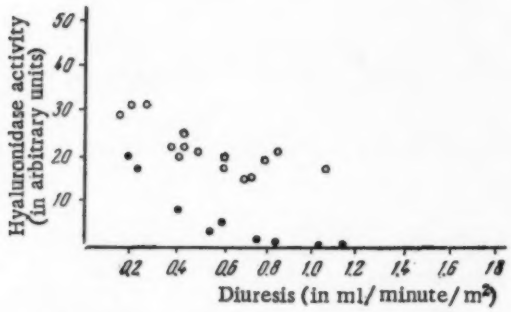

Fig. 1. Changes in the hyaluronidase activity of the urine in a dog poisoned with uranium nitrate; ● – hyaluronidase activity of the urine before poisoning; ○ – hyaluronidase activity of the urine in the same dog 1 week after poisoning.

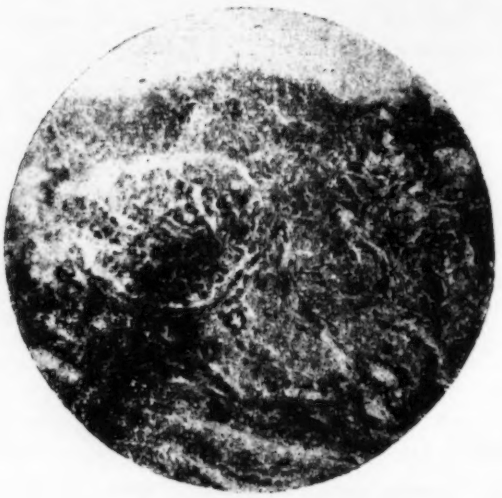

Fig. 2. Section through kidney of patient with corrosive sublimate poisoning (magnification 280 times).

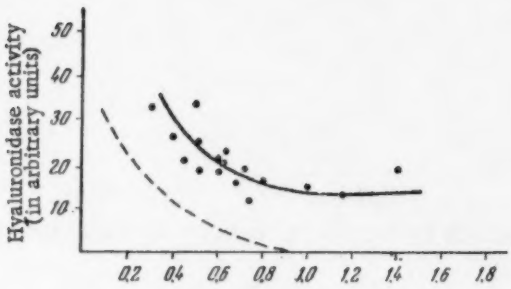

Fig. 3. Hyaluronidase activity of the urine in patient with corrosive sublimate poisoning. Dotted line shows curve of hyaluronidase activity against diuresis for healthy human subject.

SUMMARY

In kidney damage in man due to corrosive sublimate poisoning, the activity of hyaluronidase in the urine is considerably increased. The same increase in the enzymatic activity is observed in urine of dogs which were subjected to the effect of uranium salts. It is quite probable that the increased hyaluronidase activity in urine is due to the destruction of the elements of kidney tissue which liberate the enzymes in necrotic nephrosis.

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CENTRAL NERVOUS CHANGES IN EXPERIMENTAL TETANUS AND THE MODE OF ACTION OF THE TETANUS TOXIN

COMMUNICATION I. IRRADIATION OF THE EXCITATION ON STIMULATING THE TETANIZED LIMB

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According to work done up to the present time, the symptoms of tetanus are due to changes in central nervous structures, and in particular the motor neurons [2-6,9-11,17,21,26,27]. According to one theory of the pathogenesis of tetanus, the Cl. tetani toxin enters the central nervous system along the motor nerve fibers and infects the motor neurons with which it first comes in contact [16,19]. Opponents of this theory, who maintain that there is a hematogenous spread of the toxin into the central nervous system agree that the motor nerve cells of the anterior horns of the spinal cord are first affected by the toxin [8]. Recently Eccles and his coworkers (Brooks, Curtis and Eccles, [10]), have concluded from electrophysiological investigations, that the tetanus toxin has a selective action on the synapses which terminate round the motor neurons and which normally mediate inhibitory effects. These experiments, as well as the earlier physiological experiments of other authors were carried out using a local tetanus.

Nevertheless a study of the symptoms of tetanus, particularly at the stage when the condition becomes generalized, gives reason to suppose that the pathogenic mechanism of this disease is more complex than has been supposed up to the present time.

The present work was undertaken with the object of studying certain functional nervous changes in experimental tetanus with a view to elucidating the mechanism of action of the tetanus toxin. In the present communication a study is made of the phenomena which we have found in investigating the clinical condition of ascending generalized tetanus in white rats.

EXPERIMENTAL METHOD

The experiments were carried out on white rats of weight 130-165 g. Dry tetanus toxin series 580 IEM AMN was used, and was diluted with glycerin (pH 6.9) and physiological saline; the necessary dilution (1 MLD for rats weighing 130 g. contained 0.05 ml of the solution) were made prior to the experiment. The toxin was given in two injections – into the muscles of the left lower leg and left thigh. At the same time, in order to block or attenuate any action of the tetanus through the bloodstream,* .025 units of the antiserum (diaferm 3

* Preliminary experiments showed that in these conditions the phenomena described below were shown more clearly.

of the IEM AMN was given intravenously into the lower right leg. The potentials were led off to a two channel amplifier system OKB of the AMN, the output of which was taken to a Siemen's string oscillograph. Muscle potentials were picked up with needle electrodes. An electronic stimulator was used to apply stimuli to the central ends of the nerves and to the spinal roots, in order to determine the levels of the convulsive reaction; the stimulator had a potential divider at the output and delivered square wave stimuli at a frequency of 50 cps and with a duration of 0.5 msec; the value of the stimuli was determined with a cathode ray oscillograph. Platinum stimulating electrodes were used. Dissection and division of the nerves and nerve roots was carried out under brief ether anesthesia. The stimuli were applied when the animal had recovered from the anesthetic. Where any trauma of the spinal cord was suspected, the animals were not used for the experiment. The spinal roots were exposed subdurally and were cut at their exit from the vertebral canal, and this gave a sufficiently long central end, so that the distance from the electrodes to the spinal cord was more than 1 cm length. The lumbar enlargement was exposed as little as possible. The sciatic nerves were cut in the region of the popliteal fossa before branching. Besides the usual section of the femoral and obturator nerves in the region of the groin, a high section was also made in the region of the lumbar plexus, so as to exclude the possibility of anastomoses with the sciatic. All branches of the latter from where it was sectioned, to the lumbar plexus were cut. When registering the potentials, a stimulus marker was included automatically when the stimuli were applied.

EXPERIMENTAL RESULTS

The present work is devoted to the investigation of a phenomenon which we discovered: that during the development of a generalized tetanus of the ascending type, i.e., when there is at first a local tetanus of the limb which received the tetanus injection, and then a segmental and a general tetanus, at the stages when the condition has become generalized, convulsions of the whole body may be induced by applying the stimulus to the hind limb which received the toxin, (in future we shall refer to this as the tetanized limb). When the stimulus is applied to other parts of the body, including the opposite limb, either no convulsions, or very weak ones result.

This phenomenon is not shown in white rats only. Experiments have shown that it can be observed in other animals, e.g. in mice, rabbits, and in many cases in cats, after injection of corresponding doses of toxin. It may therefore be regarded as one of the characteristic signs of generalized ascending tetanus.

Investigation of this phenomenon has shown that provocation of convulsions by applying stimuli to the tetanized limb (e.g. by forced bending, or pressing on the foot or muscles of the leg etc.) is possible in the early stages, when stimuli applied to other places have no effect. As generalized tetanus advances the convulsions evoked by the stimulus to the tetanized limb become stronger and more prolonged: the limbs are extended, the tail raised, the muscles of the trunk contract, the head is thrown back in opisthotonus, the jaws are tightly clenched, the eyes half close, breathing is interrupted, and the animal frequently utters a groan. At the height of the generalized tetanus, a convulsive attack may be induced by even a mild bending of the tetanized limb or of one of its digits or by pressure on the pads of the feet with forceps. At the same time the convulsions evoked by similar stimuli applied to the other limbs, are less well shown, less prolonged and sometimes do not occur at all.

Electrical recordings made at various stages in the development of generalized tetanus confirmed what has been said above, and showed that both proprioceptor stimuli (stretching or compressing the muscles) and exteroceptor pain stimuli (pinching folds of skin or the pads of the feet with forceps) entering the central nervous system from the tetanized limb cause a very considerable increase in electrical activity with the generation of large potentials in various muscles of the trunk, neck and limbs (Fig. 1a and Fig. 2a, b). This activity coincides with the development of generalized convulsions. The electrical activity does not subside immediately the stimuli are withdrawn, but continues for a while, gradually decreasing, and the length of time for which the activity continues is greater the more severe the general picture of generalized tetanus.

When the same stimuli under the same experimental conditions are applied to the opposite limb, either there is no increase in electrical activity (Fig. 1,b and Fig. 2,b), or else there is a negligibly small increase.

To explain this phenomenon, it was at first supposed that the stimuli entering the central nervous system from the receptors of the tetanized limb were, from the onset, more powerful than those from the opposite side. Thus, for example, stretching the contracted muscles of the tetanized extremity should cause a more effective proprioceptor stimulation than stretching the less rigid muscles of the opposite side. Also, according to many others [20,25], tetanus toxin increases the excitability of the proprioceptors. Alternatively however, it is possible that the phenomenon is due to changes in the central and not in the peripheral nervous system.

To investigate this the femoral and obturator nerves were cut at a high level and the central ends of the sciatic nerve of the tetanized and of the opposite limb were stimulated. By this means any additonal proprioceptor stimulation was avoided. The results of these experiments are shown in Fig. 3 and it can be seen that the convulsion threshold for stimulation of the central end of the sciatic of the tetanized limb is several times lower than that for the opposite extremity, and that in the latter case the convulsions were less intense and sometimes poorly shown.

These results show that the facilitation of the induction of generalized convulsions from the tetanized side are due primarily to central nervous mechanisms. However, peripheral mechanisms may play a subsidiary part in this phenomenon.

To explain this effect, in view of the indication of the selective action of the toxin on the motor neuron region, it must be supposed that there is a considerable antidromic effect from the motor neurons supplying the tetanized limb, spreading into other reflex arcs.

One of the ways in which inhibition of the anterior horn cells is effected is through the interneurons being activated from stimuli passing along the recurrent motoneuron collaterals [12], as well as along the afferent fibers [15]. These cells enable the motoneurons to influence each other so that stimulation of one causes an inhibitory effect on its neighbors. Since Sherrington [7], on the basis of the observed changes in reciprocal innervation in tetanus advances the idea that tetanus may not only suppress the inhibitory action, but may change it into a stimulating one, we may suppose that such a system might mediate this transformation. There may be other mechanisms of the spread of the excitation from the motoneurons of the tetanized limb to other reflex arcs, and this may involve the system of interneurons [15].

To test this hypothesis we carried out experiments with antidromic stimulation of the motoneurons of the infected segments of the spinal cord by stimulating the central ends of the corresponding motor roots.

The results of these experiments are shown in the Table. Here it can be seen that in no case did the antidromic stimulation cause a convulsion. Even with powerful stimulation it was impossible to induce any reaction in the animal. Nevertheless, a relatively mild stimulus to the sensory roots of the same segments causes the typical convulsions. Thus in tetanus there are no abnormal antidromic effects resulting in stimulation of the motoneurons innervating the tetanized limb and consequently the induction of convulsion when a stimulus is applied to it cannot be due to any such effect. Our results agree with those of Eccles and his co-workers [10] who failed to find any transformation of inhibitory effects into excitatory stimuli in localized tetanus.

The results reported here lead us to suppose that the functional changes resulting in convulsions induced by the stimulation of the tetanized limb occur in the synapses formed by the afferent and interneurons of the reflex arcs which originate in the receptors of the tetanized limb. The destruction of some mechanism of restraint in these synapses allows the impulses originating in the periphery to spread and so to pass to other reflex arcs. The extent to which this excitation will spread depends on the extent to which reflex excitability is increased, i.e. the extent to which the inhibitory mechanisms of the various nervous pathways are suppressed, and on the number of such pathways involved.

Since at the onset of generalized ascending tetanus the functional changes occur chiefly on the side of injected toxin, and are absent or weak in other reflex arcs, stimuli entering the central nervous system from other receptor fields are to some extent suppressed in the synapses of the corresponding afferent neurons and interneurons and so do not attain the threshold value required for the induction of a generalized convulsion. To induce the latter the strength of the stimulus must be increased, i.e., the restraining effect of the synaptic mechanism must be overcome. As the condition spreads, inhibitory effects are reduced, the general reflex excitability is increased, and the threshold for the convulsive reaction evoked by stimulation of other receptor

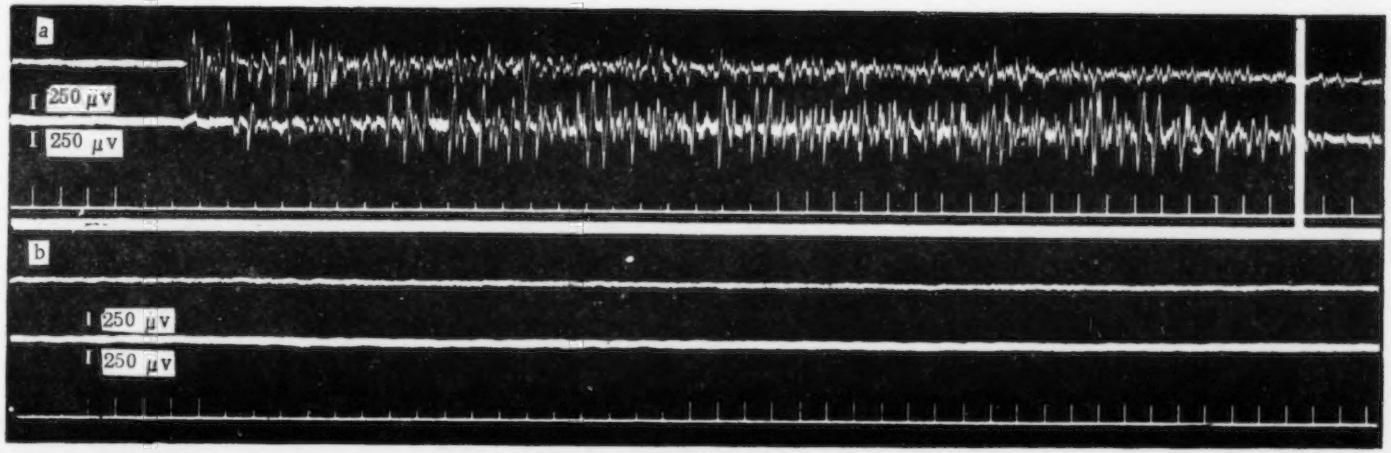

Fig. 1. Electrical activity in the triceps muscle of the left (upper oscillograph tracing) of the anterior roots induced by gentle flexion of the left (tetanized, a) and right (b) foot.
Upper oscillogram restricted to 6.9 cm (0.3 seconds). End of selected portion shown by white line across tracing. Time marking–20 msec. Reduction in size of time markings indicates period of pressure on foot. Experiment of 1/3/1955. Rat No. 5. Clearly shown segmental tetanus, increase of general excitability.

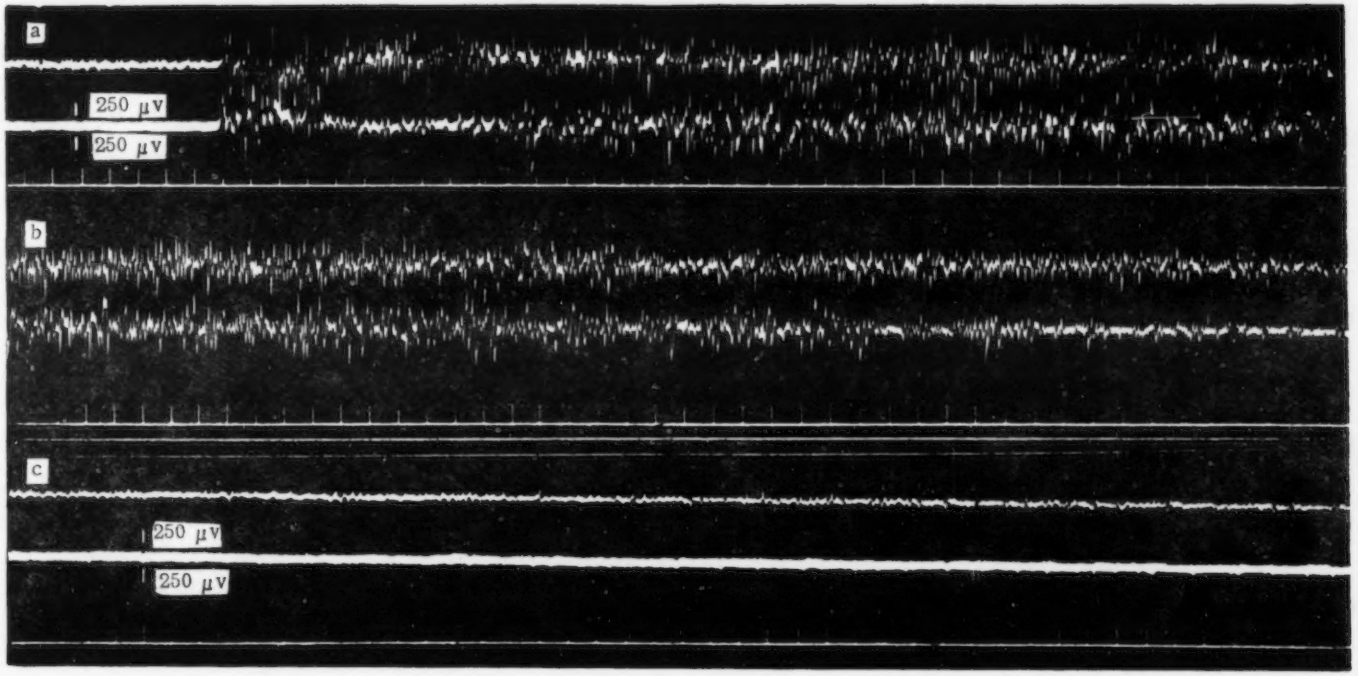

Fig. 2. Electrical activity in the dorsal neck muscles (upper tracing of each pair) and in the posterior group of muscles of the right thigh (lower tracing of each pair) on gentle flexion of the left (a and b) and right (c) foot.
Oscillogram b is the continuation of a, and is shortened to 3.5 cm (0.17 seconds). Other curves as in Fig. 1. Same experiment.

fields (e.g. that of the opposite limb) are reduced (see Fig. 3). At the peak of the tetanus even the weakest stimulus may induce a convulsive attack.

It was pointed out above that the duration of the convulsions induced by stimulation of the tetanized limb increases according to the severity of the tetanus. The electrical activity in the various muscle groups, which develops during the convulsions and indicates stimulation of the motoneurons, does not cease at the end of stimulation, but continues for a certain time and gradually fades away (see Fig. 1, a and Fig. 2, a and b). Experiments have shown that the after discharge is not caused by additional stimuli originating from the periphery, as similar effects are found in experiments where the central ends of the sciatic nerve are stimulated after high section of the other nerve trunks. It follows from the work of Eccles and his co-workers [10] that the synapses on the motoneuron cells do not initiate this after discharge. When it is realized that the length of the after discharge of the motoneurons depends chiefly on the number of interneurons which are involved and which form closed self-excitatory loops [1,13,14,15,18,22], it follows that the number and the degree of involvement of the interneurons increases according to the severity of the tetanus.

The above results give reason to suppose that the action of the tetanus toxin is not confined to the motoneurons, but spreads through the synapses to affect other reflex arcs. In this respect, the mechanism of action of tetanus toxin closely resembles that of strychnine poisoning.

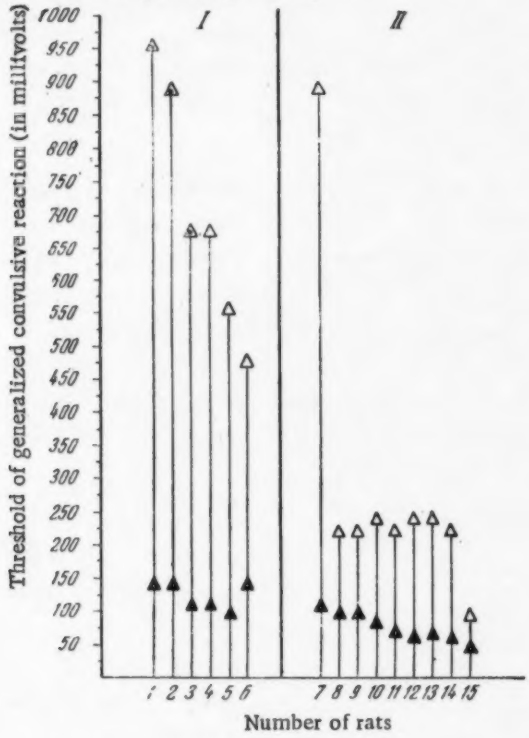

Fig. 3. Thresholds of generalized convulsive reaction caused by stimulating the central ends of the left (tetanized limb) and the right sciatic nerves.

▲) left sciatic nerve; △) right sciatic nerve. The sign ▲ indicates that stimulation of the right sciatic caused no definitive convulsions at the indicated threshold or at higher levels of excitation. I and II) stages in the development of generalized tetanus. I) first signs of segmental tetanus, increased general excitability; II) clearly shown or completely developed segmental tetanus, great increase in general excitability, spontaneous convulsions in some of the rats. In Rat No. 7 there was a clearly shown segmental tetanus, but a relatively small increase in general reflex excitability.

Reactions of Rats Infected with Tetanus Toxin to Stimulation of the Sensory and Motor Spinal Roots Innervating the Tetanized Limb

Number of rats	Weight in grams	Dose of toxin in ml	Day of illness	Description of condition	Roots stimulated		Strength of stimulus (in mv)	Reaction
1	165	0.2	3	Signs of segmental tetanus. Increase of general excitability.	Sensory	L_5+L_6	100	++
					Motor	L_6	950	—
2	165	0.2	3	The same	Sensory	L_5+L_6	145	++
				" "	Motor	L_6	950	—
3	160	0.2	4		Sensory	L_5+L_6	70	++
				" "	Motor	L_5+L_6	950	—
4	165	0.2	5		Sensory	L_6	70	++
					Motor	L_6	950	—
5	165	0.2	3	Signs of segmental tetanus. Considerable increase in general excitability, spontaneous convulsions.	Sensory	L_5+L_6	45	+++
					Motor	L_5+L_6	950	—
6	165	0.2	3	The same	Sensory	L_5+L_6	45	+++
					Motor	L_6	950	—
7	165	0.2	4	Segmental tetanus. Increase in general excitability.	Sensory	L_5+L_6	60	++
					Motor	L_5+L_6	950	—
8	165	0.2	4	The same	Sensory	L_6	60	+++
					Motor	L_6	950	—
9	160	0.2	3	Segmental tetanus. Considerable increase in general excitability, spontaneous convulsions.	Sensory	L_5+L_6	50	+++
					Motor	L_5+L_6	950	—
10	165	0.2	3	The same	Sensory	L_5, L_6	50	++
					Motor	L_5+L_6	950	—
11	165	0.2	3	" "	Sensory	L_5+L_6	45	++
					Motor	L_5+L_6	950	—
12	165	0.2	3	" "	Sensory	L_5, L_6	50	++
					Motor	L_5+L_6	950	—
13	165	0.2	3	" "	Sensory	L_5, L_6	50	+++
					Motor	L_5+L_6	950	—
14	165	0.2	4	" "	Sensory	L_5+L_6	45	+++
					Motor	L_6	950	—
15	165	0.2	6	" "	Sensory	L_5, L_6	40	+++
					Motor	L_5+L_6	950	—

Note: $L_5 + L_6$ indicates simultaneous stimulation of the two roots; L_5, L_6 – separate stimulation of each; ++ strong generalized convulsions; +++ very strong generalized convulsion; — no convulsions.

SUMMARY

Experiments were performed on white rats, affected by ascending general tetanus. The general convulsions were provoked with great ease by applying stimulation to the extremity into which a lethal dose of tetanus toxin had been introduced. This phenomenon was not connected with increased excitability of extero- and proprioceptors and cannot be explained by the functional changes of the synapses, which have an inhibiting effect on the motor cells of the anterior horns of the spinal cord. The data which were obtained allow us to conclude that the effect of tetanus toxin is not limited to the area of motoneurons but that it, likewise, spreads to the synaptic formations of other portions of the multineuronic reflex arcs.

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THE MECHANISM OF DYSENTERY INTOXICATION

COMMUNICATION III. SPECIFICITY OF ACTION OF THE ENTERIC GROUP OF TOXINS ON INTESTINAL CHEMORECEPTORS

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The question of specificity of toxin action is a controversial one. A.D. Ado and collaborators and other [1,2] consider that toxins possess no specific properties in their action on the nervous system. A.D. Speransky and collaborators, H. Raskova and collaborators and others [9,10,14] maintain, on the other hand, that the relation of various toxins to nervous elements is specific.

As already reported [7,8], application of dysentery toxin to the mucosa of a perfused intestinal loop leads to changes in the intensity of arterial blood pressure reflexes to stimulation of receptors by nicotine.

METHODS

52 experiments were performed on cats. As in the previous experiments [7,8], chemoreceptor activity was studied on a portion of the intestine which had been isolated from the general circulation. The order of experimental procedure was the same as before. Exotoxins of Shiga bacilli and Staphylococcus aureus and endotoxins of Flexner bacilli C and E. coli was used.

RESULTS

In the first series of experiments (17 experiments) the E. coli toxin was studied. Being a constant inhabitant of the intestine E. coli may, under certain conditions, give rise to an illness resembling dysentery [5,12, 13]. According to observations by Vincent [15] intravenous injection of E. coli toxin into rabbits led to their death with manifestations of paralysis starting in the hind limbs. At autopsy severe myelitis with extensive cellular degeneration in the anterior and posterior horns was found together with signs of marked enteritis.

This gave rise to the question whether E. coli toxin resembled the Shiga bacillus toxin in its effect on receptors.

Twelve cats in this series were given E. coli toxin subcutaneously in amounts of 0.5-3 LD(mouse) per 1 kg body weight. Only two of these were very ill on the 3rd day of intoxication, and the animals died during preparation for the experiment. The remaining 10 cats did not differ in their behavior from healthy ones. These were subjected to experiments on the 1st and 2nd day of intoxication (8 experiments), 7th day (1 experiment) and 11th day (1 experiment). No autopsy was performed,but when the intestinal loop was being prepared it was seen that the small and large intestine mucosa was hyperemic and that there was enlargement of mesenteric lymph nodes; dehydration of tissues was noted in two cases.

Application of E. coli toxin to the mucosa of the perfused intestinal loop (2-4 mouse LD) led to reduction of intensity of reflex arterial blood pressure reactions to intravascular introduction of nicotine (7 experiments on the 1st, 2nd and 7th day of intoxication). Only in one out of seven experiments restoration of the intensity

of reflexes was observed after 8 minutes; enhanced reflexes were noted in two experiments performed by the end of the 1st day of intoxication. No changes in reflex were obtained on the 11th day of intoxication.

Figure 1 presents kymograms of one of such experiments. Introduction of E. coli toxin (↓) into the intestinal lumen reduced the intensity of reflex reactions to nicotine from 10 to 6 mm in 1 minute. After

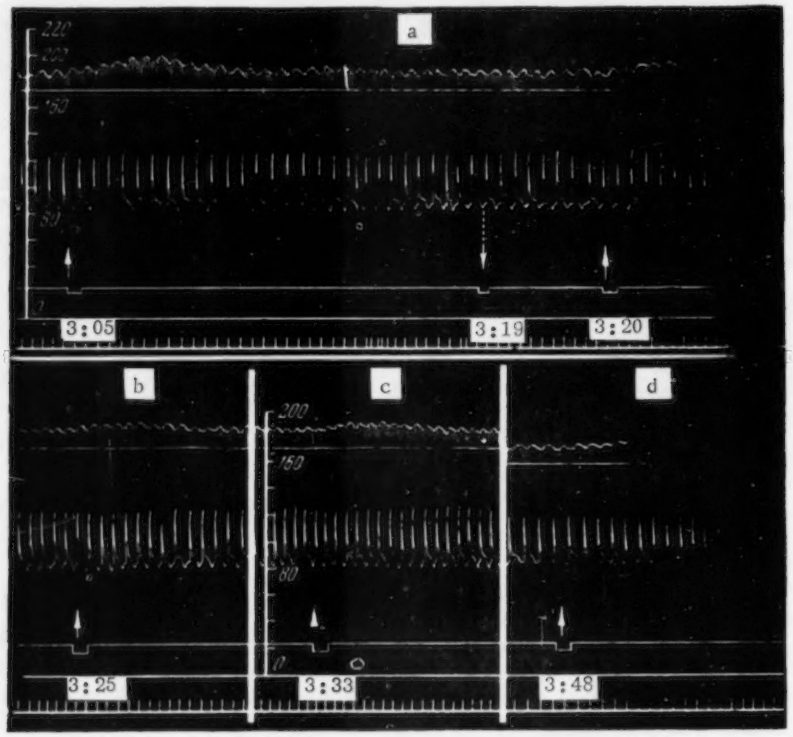

Fig. 1. Nature of reflex reaction to nicotine in cat upon introduction of E. coli toxin (0.6 LD per 1 kg body weight) (second day of intoxication).
Records from above down: arterial blood pressure, initial level of arterial blood pressure, respiration, stimulation mark (↓ – introduction of toxin, ↑ – introductionof 10 γ nicotine), manometer base line, time marker (2 seconds).

6 minutes the height to which arterial blood pressure rose following introduction of nicotine was only 4 mm (d). After 29 minutes the intensity of reflex reaction remained the same (f). The reflexes did not return to the initial level.

In control experiments introduction of E. coli toxin into the lumen of the perfused intestinal loop of seven healthy animals caused no change in the intensity of pressor reactions in response to nicotine stimulation of intestinal chemoreceptors.

This series of experiments thus indicates that E. coli toxin acting on an animal affected by the same toxin alters the chemoreceptor activity of the intestine similarly to Shiga bacillus toxin.

In the second series (14 experiments) a study was made of chemoreceptor activity of the intestine under the influence of Flexner bacillus C, a representative of the so-called weakly toxic group of dysentery pathogens.

The available toxin was weak (0.5-1 mouse LD per 1 ml solution), which could not but affect the extent of intoxication development, the morphologic picture and the influence of the toxin on interoceptive

reflexes. The general condition of these animals was considerably better than of those under the influence of Shiga bacillus toxin. In 5 out of the 14 experiments in this series no changes in the intensity of reflex reactions were obtained upon stimulation of the intestinal mucosa by the toxin. At autopsy only slight hyperemia of intestinal mucosa was found in all 5 animals.

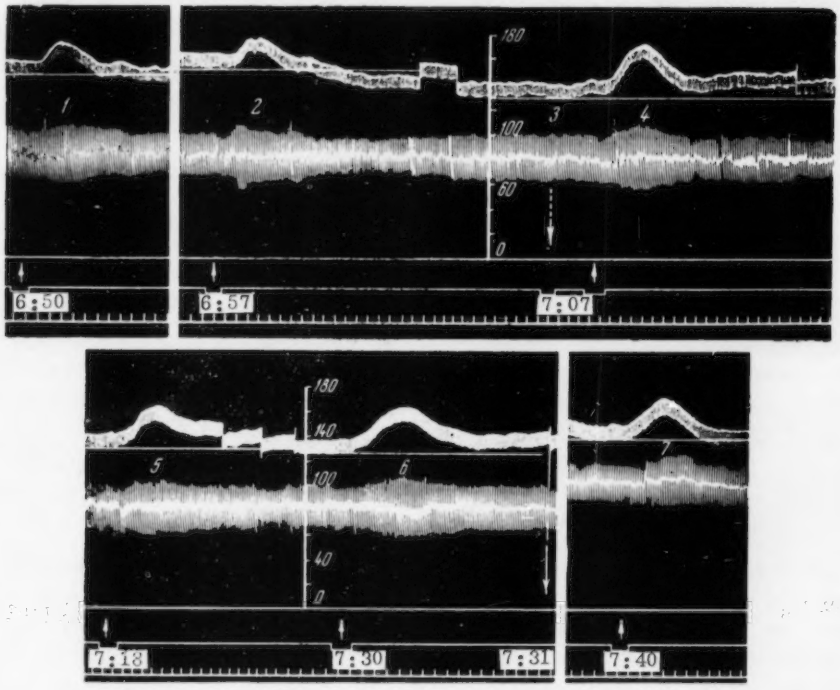

Fig. 2. Nature of reflex reactions to nicotine in cat upon introduction of Flexner toxin (0.85 LD per 1 kg body weight) 5 hours prior to experiment.
Records from above down: arterial blood pressure, initial level of arterial blood pressure, respiration, manometer base line, stimulation mark (↓ – introduction of toxin, ↑ – introduction 10 γ nicotine), time marker (5 seconds).

The experiments were performed 4-6 hours after subcutaneous administration of the toxin and on the 4th day of intoxication. In the first group of experiments in this series application of Flexner dysentery toxin (3-5 mouse LD in 1.5-2.5 ml solution) to the mucosa of the perfused intestinal loop led to enhancement of arterial blood pressure reflexes to nicotine stimulation in three experiments. In one case no change in the reflex was obtained.

At autopsy marked hyperemia of the intestinal mucosa especially of the small intestine, was observed. The results of the second series of experiments are presented in Fig. 2. Two and one-half hours after subcutaneous injection of the toxin (↓) the cat began to vomit repeatedly (5 times during 30 minutes). Motor restlessness appeared. Introduction of the toxin into the intestinal lumen was accompanied by a persistent enhancement of reflexes from 14 to 30 mm. After 10 minutes the reflex level dropped to 26 mm. Deepening the narcosis (↓) did not alter the intensity of the reflex reactions.

The second group of experiments in this series was performed on the 4th day of intoxication. In 5 out of 10 experiments introduction of Flexner bacillus toxin into the intestinal lumen elicited reduction of pressor reactions to nicotine with their restoration within 15-60 minutes. In 4 experiments no changes in reflexes were observed. In one experiment toxin stimulation of intestinal mucosa receptors increased the intensity of

reflexes to nicotine by 6 mm, compared with the initial level, in the course of 20 minutes. At autopsy the animals in which there was a lowering of reflex reaction showed macroscopic edema of the tissues of both the small and the large intestine with hyperemia and hemorrhages in the mucosa. A large amount of mucus was found in the intestine. No mucosal necrosis was found.

Comparsion of the experimental results obtained with Flexner bacillus endotoxin and Shiga bacillus exotoxin allows the conclusion that changes in intestinal chemoreceptor activity produced by these two toxins are analogous.

The third series of experiments (6) made use of Staphylococcus aureus toxin. It is well known that Staphylococcus aureus does not develop in the intestinal tract but when ingested with food may cause severe food poisoning with manifestations of gastritis and enteritis [3,4,6,11].

In this series the experiments were performed on animals previously poisoned by Staphylococcus aureus toxin. Stimulation of the mucosa of the perfused intestinal loop by Staphylococcus aureus toxin under conditions of intoxication did not affect the character of interoceptive reflexes in any of the 6 experiments (Fig. 3).

The experimental results in this series indicated that under conditions of staphylococcal intoxication application of Staphylococcus aureus toxin to the mucosa of the perfused part of the intestine left the intestinal chemoreceptor activity unchanged.

The results of the three series of experiments and those of our previous experiments with Shiga bacillus toxin [7] permit the conclusion that intestinal receptors possess particular sensitivity to pathogen toxins.

The fourth series (15 experiments) was concerned, on the one hand, with the effect of dysentery toxin on intestinal chemoreceptors under conditions of staphylococcal intoxication and, on the other hand, with the effect of staphylococcal toxin on intestinal interoceptive reflexes under conditions of dysentery intoxication produced by Shiga toxin.

1.2-1.5 ml Staphylococcus aureus toxin (16 thousand hemolytic units per 1 ml) was given to five animals 24 hours prior to the experiment. During the experiment dysentery toxin (60-120 LD) was applied to the mucosa of the perfused part of the intestine. In none of the 5 experiments was there any change in the nature of intestinal receptor reflexes to nicotine.

In the remaining 10 experiments intoxication was produced by subcutaneous injection of Shiga bacillus toxin (15-30 LD per 1 kg body weight).

Five experiments were performed on the 2nd day of intoxication. Introduction of Staphylococus aureus toxin into the intestinal lumen did not alter the intensity of reflexes to nicotine in any of the 5 experiments. The other 5 experiments were performed on the 4th day of intoxication. The animals were very ill; the arterial blood pressure and intensity of arterial blood pressure reflex reactions were diminished in the course of the experiment. No clearcut results could be obtained in these experiments.

The data of the last series of experiments convinces us that against the background of dysentery intoxication, the sensitivity of receptors is altered only with respect to dysentery toxin.

Staphylococcal intoxication, on the other hand, evidently does not enhance the intestinal receptor sensitivity to dysentery toxin.

The results of these experiments and those with the Shiga bacillus toxin [7] show that toxins of representatives of the enteric group of microbes, both pathogenic (Shiga and Flexner bacilli toxins) and conditionally pathogenic (E. coli toxin), exert an analogous influence on the intestinal chemoreceptors. Staphylococcus aureus toxin, a nonspecific intestinal stimulant, does not exhibit such influence either in dysentery or staphlococcal intoxication.

In acknowledging the existence of specific sensitivity of receptors to certain toxins we join the view held by O.Ya.Ostryi [9] who considers that in the process of phylo- and ontogenesis certain receptors which had been in predominant contact with certain stimuli have become the objects of one or other type of stimulation. Evidently that is the reason underlying the fact that toxins of various representatives of the enteric group possess common properties with respect to their action on intestinal receptors.

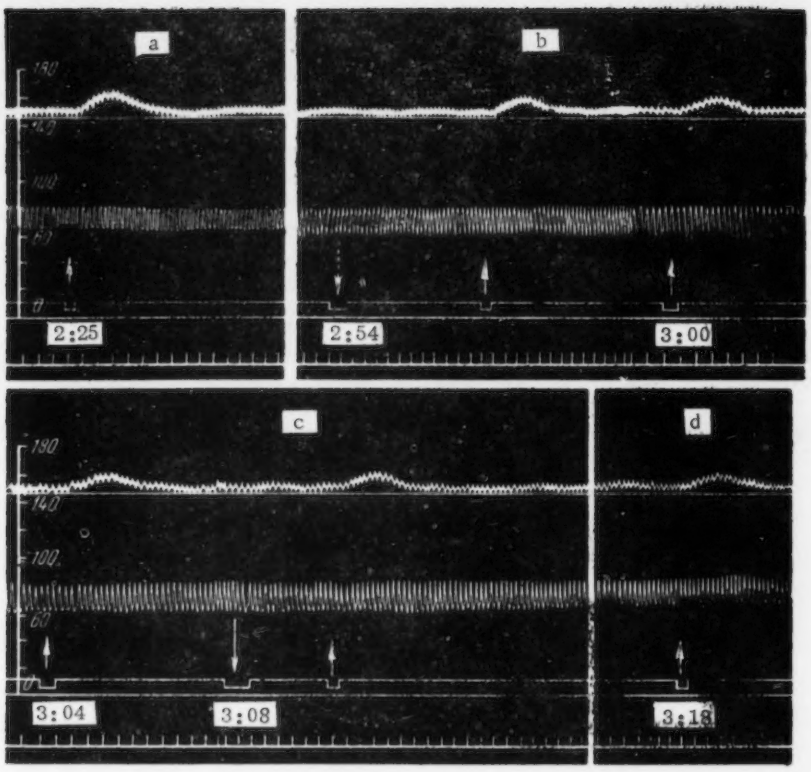

Fig. 3. Nature of reflex reactions to nicotine in cat in experiment with preliminary introduction of Staphylococcus aureus toxin (0.2* per 1 kg body weight). Records from above down: arterial blood pressure, initial level of arterial blood pressure, respiration, stimulus mark (↓↓ – introduction of 0.5* staphylococcus, ↑ – introduction 1γ nicotine), time marker (5 seconds).

SUMMARY

A comparison was made of the effect of exotoxins of the Shiga bacillus , endotoxins of Flexner bacilli and E. coli against the action of exotoxin of Staphylococcus aureus on the intestinal chemoceptors. The data which were received support the view of the presence of specific sensitivity of intestinal receptors to the toxins of pathogenic and conditionally pathogenic microbes of the intestinal group.

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THE EFFECT OF CORTISONE UPON TYPHOID INTOXICATION IN RABBITS

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During infectious illnesses medicines not possessing antibacterial actions are used at times as, for example, antihistamines and hormones, the goal sought being the alleviation of the manifestations of intoxication or the blockade of tissue pathological reactions.

From the literature, it is known that cortisone exacerbates the clinical course of tuberculosis [9,15], spirochetoses [16], mycoses [20] and many other infections. We established that cortisone speeds the clinical course of rabbit pneumococcal infections, hastening their death [4]. The question arises as to the reason for this negative influence of the cortisone upon so many infections: is it that it depresses those factors which act as barriers to the multiplication of the infectious agents within the organism or is it that it suppresses those mechanisms which prevent the intensification of developing intoxications?

Z.A. Ertuganova showed that cortisone suppresses the ability of the endothelial-macrophagic system to have its cells destroy the engulfed bacteria [5]. This fact may be one of the reasons why cortisone hastens the course of the infection and hasten the dissemination of the bacteria. Alongside with this observation, some authors have observed that cortisone lowers the febrile response in patients with typhoid fever and in animals in response to typhoid vaccine [13]. V.I. Goncharova showed that cortisone diminishes the percentage of deaths in mice and prevents the death of rats during the course of typhoid intoxication [1]. The author attributes this fact to increased resistance to the bacterial intoxication on the part of the organism receiving cortisone.

Having been convinced that cortisone intensifies the severity of rabbit pneumococcal infection [4], we set ourselves the goal of determining the effectiveness of the preparation in a pathological situation where there was only the factor of intoxication, the living microbial agent having been excluded. For this purpose we used the method of producing typhoidal intoxication in rabbits.

EXPERIMENTAL METHODS

The experiments were performed on 28 rabbits weighing an average of 2.2 kg, the animals being divided into four equal groups. The first group received only typhoid vaccine, the second was given cortisone and typhoid vaccine, the third group was given cortisone only and the fourth group received physiological saline. The animals in the first two groups received in the ear veins a slow injection of warmed typhoid vaccine prepared from laboratory strain B. Typhoides abdominis #4446, the dose being 25 billion microbes per 1 kg body weight. Biddle, Sawyer & Co. cortisone acetate was used. The cortisone was given intramuscularly as 5 mg per 1 kg body weight, this dose being repeated twice daily until the death of the animal. The first injection was given 30 minutes prior to the injection of the typhoid vaccine. The physiological saline was given in the amount of 0.1 cc and administered in the same sequence as cortisone.

Blood sugars in the rabbits were done by the method of Hagedorn-Jensen; adrenalin in the blood and adrenals by the Utevsky method; while ascorbic acid content of blood and adrenals was determined by titration with 0.02% solution of 2, 6-dichlorphenolindophenol.

The blood for the determinations was taken from the ear vein of the fasting animal prior to injection of cortisone or typhoid vaccine, 24 hours after the injection of the vaccine if the animal was still alive and also at the moment of death. Two rabbits in the first group and 3 in the second which were in very poor condition were killed by means of air emboli. The rabbits of the 3d and 4th group were killed by means of air emboli in the same time intervals at which the animals of the 2d group died.

Immediately on the death of the animal , the adrenals of the animal were taken out and weighed, after which they were ground in quartz sand and prepared for further studies by having the proteins extracted with the aid of 10% solutions of trichloroacetic acid.

EXPERIMENTAL RESULTS

The experiments of this investigation indicate that cortisone delays rabbit deaths resulting from typhoid intoxication an average of 38 hours (Table 1). It follows that cortisone did not suppress the mechanisms producing resistance to typhoid intoxication.

TABLE I

Survival Rate of Animals Subjected to Typhoid Intoxication and Both Treated and Untreated with Cortisone

Preparation introduced	Total number of rabbits	Number of rabbits dying after								Number of rabbits sacrificed after			
		8	10	14	15	23	40	44	99 hrs.	32	48	89	90 hrs.
Typhoid vaccine	7	1	1	1	2	0	0	0	0	0	1	1	0
Cortisone and typhoid vaccine	7	0	0	0	0	1	1	1	1	1	1	0	1

The normal blood sugar in rabbits is 108-117 mg %. In the first group the blood sugar level in the first 24 hours of observation remained normal.. In the rabbits of this group, dying at later times, there was noted a hyperglycemia, the concentration of the sugar rising 2- $2\frac{1}{2}$ times over the base figures reaching 267 mg %, 218 mg %.

When cortisone was administered, hyperglycemia was observed within a day of introduction of the typhoid vaccine. Only in 2 rabbits of the second group (in one, at the moment of death) did the sugar concentration in the blood not rise within the first day. Subsequently, all the rabbits developed hyperglycemia. Compared with base level, blood sugars rose $1\frac{1}{2}$- 2 fold. Giving cortisone to healthy rabbits caused blood sugars to rise $1\frac{1}{2}$-2 times as compared with beginning readings. It follows that cortisone did not substantially affect blood sugar levels produced by typhoid intoxication as it produced hyperglycemia also in healthy rabbits.

The normal rabbit blood adrenalin concentration averages 0.027 mg %, fluctuating between 0.011 and 0.036 mg %. In the first day of observation, the rabbits of the first group maintained an adrenalin level within normal limits averaging 0.026 mg %. Rabbits of this group sacrificed in the second day or later had a 2-$2\frac{1}{2}$ fold adrenalin rise averaging between 0.063 and 0.047 mg % and being in agreement with similar data for the fourth animal group. In this fourth group of rabbits all indicators remain unaltered during the course of the experiment.

In rabbits of the second group hyperadrenalinemia was observed within the first day averaging 0.049 mg %. In the second day the adrenalin concentration in the blood attained still greater figures averaging 0.063 mg %; this level was also above normal averaging 0.044 mg % in rabbits dying at later times. Cortisone injected into healthy rabbits did not produce appreciable changes in the adrenalin content of the blood.

TABLE 2

Alterations in Adrenalin Content of Rabbit Adrenals Receiving Cortisone during Typhoid Intoxication

No Group	Preparation being injected	Number of rabbits	Amount of adrenalin in the adrenals (in μg)			Weight of adrenals (in mg) per 1 kilogram body weight
			in 1 mg of tissue	in both adrenals	per 1 kg of body weight	
1	Typhoid vaccine	7	0.041±0.009	13.08±2.00	6.19±1.27	197.80±19.53
2	Cortisone and typhoid vaccine	5	0.055±0.010	14.47±2.26	8.27±1.32	176.49±22.47
		1	0.175	58.28	37.60	214.84
		1	0.143	48.76	23.56	164.73
3	Cortisone	7	0.141±0.016	26,29±3.02	12.86±1.88	94.24±15.60
4	Physiological saline (control experiment)	7	0.131±0.017	37.37±3.02	15.90±3.05	116,96± 8,22

TABLE 3

Alterations in Ascorbic Acid Content in Rabbit Adrenals Receiving Cortisone during Typhoid Intoxication

No Group	Preparation being injection	Number of rabbits	Amount of ascorbic acid in adrenals in μg			Weight of adrenals (in mg) per 1 kilogram body weight
			in 1 mg of tissue	in both adrenals	per 1 kg of body weight	
1	Typhoid vaccine	7	0.66±0.077	251.82±70.41	115.68±22.43	197.80±19.53
2	Cortisone and typhoid vaccine	7	0.79±0.15	263.27±64.28	146.55±34.22	180.28±16.37
3	Cortisone	7	1.12±0.017	215.42±40.5	108.97±17.74	94.24±15.60
4	Physiological saline (control experiment)	7	1.84±0.16	353.10±33.77	223.82±13.21	116.96± 8.22

The average data on the adrenalin determinations in the adrenals is given in Table 2. Table 2 shows that adrenalin sharply diminishes in the adrenals as a result of typhoid vaccine administration, in spite of their increased weight. The adrenalin content of the adrenals was $2\frac{1}{2}$ to 3 times less while the weight of the glands was $1\frac{1}{2}$ times greater in the animals of the first group as compared with animals of the fourth group. In 5 rabbits of the second group the adrenalin content in the ardrenals was about 2 times lower than in animals of the fourth group, although somewhat higher than in animals of the first group. In 2 rabbits of the second group the cortisone fully prevented adrenalin depletion in the adrenal glands under the influence of typhoid vaccine. The corresponding adrenalin indicators were somewhat higher in them than in the fourth group (see Table 2) which may be attributed to individual fluctuations. When cortisone is administered to healthy rabbits, the adrenalin content of the adrenals remains unchanged while the weight of the glands diminishes slightly.

It follows that introduction of cortisone during typhoid intoxication did not interfere with the rise of adrenalin in the blood and exerted no appreciable influence on the decrease of the adrenalin in the adrenal glands of the majority of the rabbits. Giving the cortisone to healthy rabbits did not materially alter the adrenalin concentration in the blood and adrenals, although slightly decreasing the weight of the glands.

The ascorbic acid concentration in the blood of normal rabbits averages 0.35 mg %. In rabbits of the first group there was observed a tendency for a rise in the concentration of the ascorbic acid. In the second group of animals the concentration of the ascorbic acid did not alter in the first 24 hours, averaging 0.33 mg % but was above normal in rabbits dying in the second day, averaging 0.97 mg % and, still later, 0.6 mg %. In the third group there was noted a rise in the ascorbic acid of the blood, being about 2 fold the base level, as well as in rabbits sacrificed in the second day and later.

The averages on the ascorbic acid adrenal gland contents are presented in Table 3. Under the influence of the typhoid vaccine the adrenal content of the ascorbic acid falls sharply. In rabbits of the first group the ascorbic acid content of the adrenal averages some 1½ to 2 times less than in rabbits of the fourth group. The adrenals of the rabbits of the second group had about the same amount of ascorbic acid as the adrenals of the rabbits of the first group. The adrenals of healthy rabbits receiving cortisone had 40-50% less ascorbic acid than the adrenals of the control animals. Thus, it appears that cortisone raised the ascorbic acid concentration in the blood and diminished the adrenal content in healthy rabbits but had no influence on the quantitative changes of ascorbic acid in the blood and suprarenals of animals during typhoid intoxication.

DISCUSSION OF RESULTS

The delay produced by the influence of cortisone in the death of the animals as a result of typhoid intoxication may be due to two reasons: decreased permeability of the walls of the blood vessels and tissues and the antihistaminic action of cortisone. This latter diminishes the permeability of blood vessel walls [2, 7] and tissues [6,8,11,12,14,17,18] which, apparently, decreases the rate at which the intravenously administered typhoid endotoxin leaves the blood to enter the tissues.

The antihistaminic action of cortisone is manifested by its ability to activate histaminase [19] and, in the opinion of some authors, by the hindering of histamin formation [10]. These important properties of cortisone determine its role in the pathogenesis of intoxication as histamin and histamin-like substances forming in the organism as a result of endotoxin action are determining factors in some of the manifestations of intoxication [3].

The influence of cortisone in preventing adrenalin loss from the adrenal glands of rabbits intoxicated with typhoid vaccine may be attributed to the antihistaminic properties of the preparation. This effect is less evident with typhoid intoxication than with pneumococcal intoxication where the cortisone completely prevents adrenalin depletion in the glands keeping the actual levels within normal limits. The differences between the influence of cortisone during typhoid and pneumococal intoxication may be explained by the fact that, in the first instance, the blood receives a lethal dose of the endotoxin and all its manifestations develop quite rapidly, within a few hours, while with pneumococcal infections the intoxications develop more slowly requiring several days. It is also quite probable that the inhibiting effect of cortisone on adrenalin depletion in the adrenal glands may be different in pneumococcal infection from typhoid intoxication because of the specific actions of the toxin.

SUMMARY

The introduction of cortisone in the dose of 5 mg per kilogram of body weight to rabbits twice a day in lethal typhoid intoxication prolonged the life of these animals up to 38 hours, on the average. However, it had no effect on the disturbance of the sugar concentration, blood level of adrenalin and the level of ascorbic acid in the blood and suprarenal glands. It had a certain retarding effect on depletion of adrenalin in the suprarenal glands.

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BIOCHEMISTRY AND BIOPHYSICS

A STUDY OF THE METABOLISM OF PHOSPHORUS COMPOUNDS IN THE HEART, WITH THE AID OF RADIOACTIVE PHOSPHORUS

COMMUNICATION III. THE EFFECT OF THE PAVLOV REINFORCING NERVE ON THE RATE OF EXCHANGE OF HIGH-ENERGY PHOSPHORUS COMPOUNDS IN THE NORMAL AND IN THE PATHOLOGICALLY ALTERED HEART

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In a previous discussion [3], we undertook the study of the effect of the cardiac reinforcing nerve, discovered by I. P. Pavlov [2], on the metabolism of adenosine triphosphate, phosphocreatine and inorganic phosphorus in the heart of healthy dogs. On the basis of the comparison of the specific activity of the indicated fractions, 15 minutes after radiophosphorus was administered intravenously under normal conditions and with irritation of the reinforcing nerve, we drew inferences as to an increase in the turnover rates of ATP and phosphocreatine in the heart, under the influence of this nerve.

However, those data, obtained within a given period after the administration of P^{32}, did not preclude the possibility of a different pattern of dynamics operating in the penetration of P^{32} into the compounds under discussion, under normal conditions and upon irritation of the reinforcing nerve, and more precise studies of the dynamics involved were therefore in order. In accord with the above, the present discussion sets out, first of all, to determine the effect of the reinforcing nerve on the rate of exchange of ATP and phosphocreatine in the heart, with the aid of P^{32} incorporation studies at different intervals after administration of the label, and, secondly, to find out how the effect of reinforcing nerve on the exchange of these substances varies in response to pathological conditions.

The results obtained by B. A. Vinokurov [1] are, of the data available in the literature, the ones bearing the most direct relation to the problem under discussion. The author disclosed that, on asphyxia brought about in experiments on animals by way of cessation of artificially induced respiration, and on hypoxemia, caused by forcing a gas mixture deficient in oxygen into the lungs of animals, a sharp drop-off in the positive effect resulting from stimulation of the reinforcing nerve is observed. In severe degrees of hypoxia, this effect disappears entirely, while in especially severe cases of oxygen starvation, irritation of the reinforcing branches often contributes to the onset of palpitations on the part of the ventricles of the heart.

Data on variations in the influence of the extracardial nerves on exchange processes in the heart under pathological conditions are not available in the literature, as far as we were able to determine. Of various pathological states, we considered it most interesting to make a study of the changes ensuing in the course of the turnover processes of ATP and phosphocreatine, taking place in the heart in response to reinforcing nerve actions when a myocardial infarct is induced experimentally.

EXPERIMENTAL METHOD

The experiments were carried out on 64 dogs. In all cases, the thorax was opened up while the dogs were under morphine-barbamyl* narcosis, and the cardiac reinforcing nerve was prepared with the animals under artificially induced respiration. Radioactive phosphorus was administered intravenously to some of the animals 15 minutes after this. In the other animals, myocardial infarction was brought about by ligating the left descending coronary artery, and 15 minutes after the ligature, when the electrocardiogram disclosed symptoms of acute local impairment of coronary circulation. Radiophosphorus was also administered to these animals. At preassigned intervals thereafter (5,10,15,20,25,30, and 60 minutes), an abscission of the apex of the heart was performed, while, in the infarction experiments, the section of the heart cut away contained both a portion of the ischemia and the tissue surrounding it.

The heart studies carried out in the first series of experiments took place on a background of normal performance and on irritation of the reinforcing nerve in normal animals; in the second series of experiments, on a background of normal performance and on irritation of the reinforcing nerve in dogs suffering from a myocardial infarct.

The part of the heart cut away was quickly frozen in liquid nitrogen, carefully ground to a fine power and subjected to biochemical investigation. The research technique utilized has already been described in a previous communication and will not be repeated in the present discussion. In all the experiments, arterial pressure and the electrocardiogram were recorded as a control of the functional state of the heart.

EXPERIMENTAL RESULTS

A physiological analysis of the data which we obtained revealed a considerable drop in the reinforcing effect resulting from irritation of the reinforcing nerve in response to myocardial infarction.

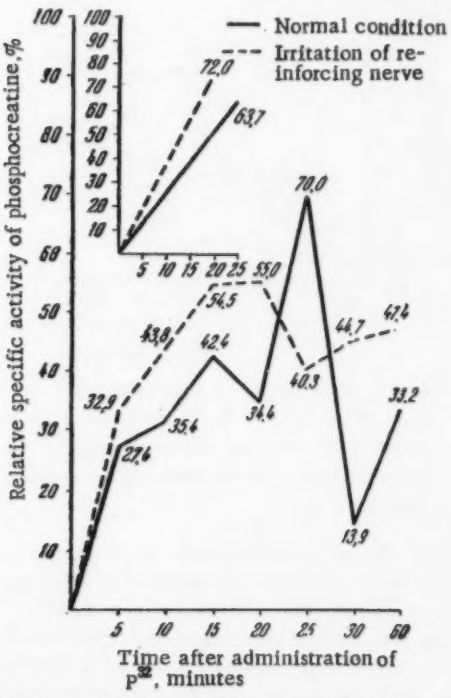
Normal condition
Irritation of reinforcing nerve
Relative specific activity of phosphocreatine, %
Time after administration of P32, minutes

Fig. 1. Variations in the relative specific activity of phosphocreatine in the heart in response to irritation of the reinforcing nerve in normal dogs.

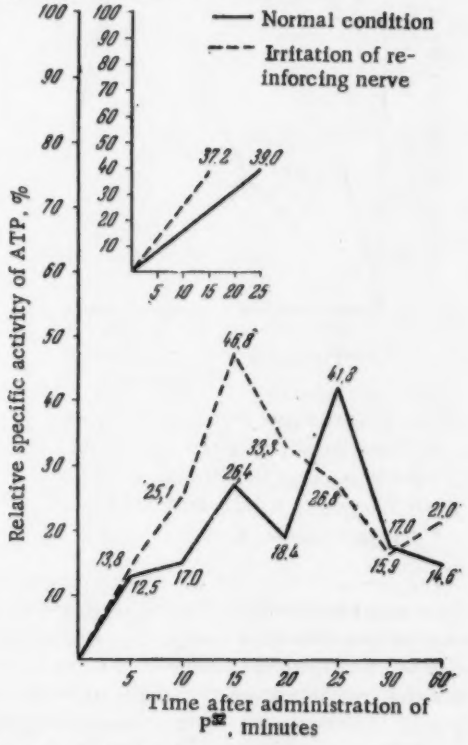
Normal condition
Irritation of reinforcing nerve
Relative specific activity of ATP, %
Time after administration of P32, minutes

Fig. 2. Variations in the relative specific activity of ATP in the heart in response to irritation of the reinforcing nerve in normal dogs.

* Russian trade name.

The biochemical data were first computed in terms of the specific activities of inorganic phosphorus, phosphocreatine and ATP, and plotted as curves. A comparison of the curves of the specific activities of ATP and of phosphocreatine bring confirmation to the conclusion drawn in the previous communication relating to the increases in ATP and phosphocreatine turnover in response to irritation of the reinforcing nerve. However, these findings still do not justify our inferring that the exchange rates of these substances are stepped up, since the intensified turnover rates of ATP and phosphocreatine could be the result of an exchange taking place with inorganic phosphorus at a high turnover rate. To preclude that possibility, we expressed our data in terms of relative specific activity, referring the specific activities of ATP and of phosphocreatine to the specific activity of tissue inorganic phosphorus taken as the standard. Taking into account the fact that the activity of the latter might show an increase due to blood inorganic phosphorus present in cardiac muscle, the specific activity of the last mentioned being far from uniform at different intervals after the administration of P^{32}, we first introduced into the specific activity values for tissue inorganic phosphorus the corresponding corrections. For this purpose, the specific activity of blood inorganic phosphorus was determined for all the experiments and the amount of blood present in the cardiac muscle was ascertained on the basis of hemoglobin content in the aqueous extract, by spectrophotometric measurement.

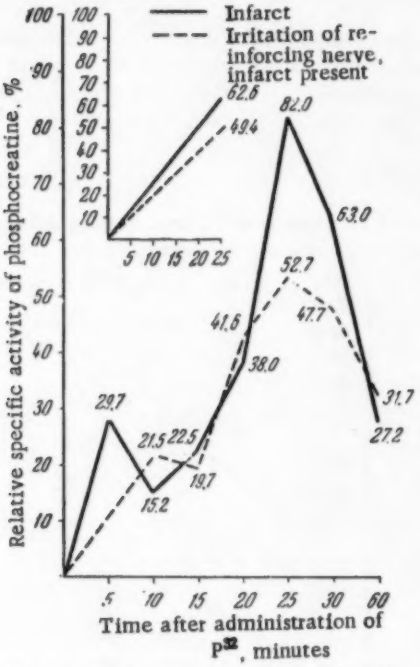

Fig. 3. Variations in the relative specific activity of phosphocreatine in the heart in response to irritation of the reinforcing nerve in dogs suffering from a myocardial infarct.

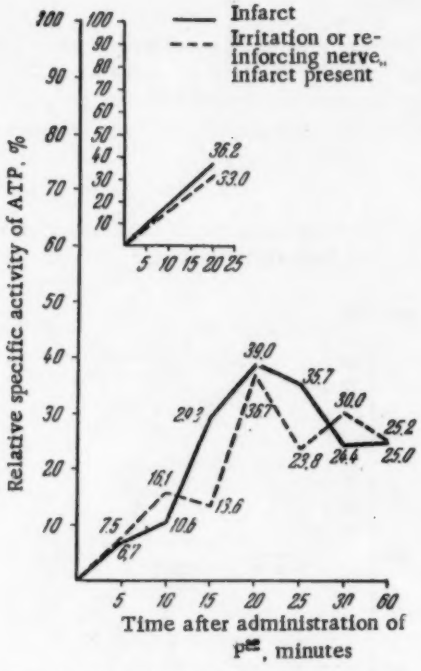

Fig. 4. Variations in the relative specific activity of ATP in the heart in response to irritation of the reinforcing nerve in dogs suffering from a myocardial infarct.

The curves obtained for the relative specific activities of ATP and phosphocreatine under normal conditions and upon irritation of the reinforcing nerve are shown in Figs. 1 and 2. The turnover rates for ATP and phosphocreatine find their expression in the slope of the curves. However, the shape of these curves, each point on which represents an arithmetic mean of determinations from several animals, renders more complicated the quantitative calculation of the general trend of the process. To facilitate the operation, we carried out a statistical treatment of the data obtained using the method of least squares, making the assumption that the rate of incorporation of P^{32} into the fraction studied per element of the curve over a range from 0 to the maximum was proportional to time. As a result of that treatment, we obtained straight lines corresponding to those elements of the curve. A comparison of the slope of those lines revealed, with greater clarity than was the case with the corresponding curves, that the exchange of ATP and of phosphocreatine, on irritation of the reinforcing nerve, underwent an acceleration.

Other measurements were obtained by irritation of the reinforcing nerve in dogs having a myocardial infarct. The curves associated with the relative specific activity of ATP and phosphocreatine and on irritation of the reinforcing nerve in dogs with a myocardial infarct are shown in Figs. 3 and 4.

A comparison of these curves and the corresponding straight lines plotted according to the method of least squares demonstrated that the rate of ATP and phosphocreatine exchange, with myocardial infarct present, not only did not increase in response to irritation of the reinforcing nerve but, conversely, showed a decline.

The absence of any increase in the rate of ATP and phosphocreatine exchange characteristic of the influence of the reinforcing nerve is evidently linked to the fact that the compensatory possibilities which exist in the heart for enhancing the exchange of those substances turns out to be exhausted when an acute impairment of coronary circulation in the heart takes place, and a subsequent stepping up of the rate of exchange of those substances, in response to irritation of the reinforcing nerve, becomes impossible.

In this manner, as a result of the disturbances occurring in the heart in myocardial infarction, cardiac muscle, exposed to the corresponding actions of the nerve, is deprived of the possibility of intensifying its contractions by virtue of the stepping up of the exchange of the basic energy sources, adenosine triphosphate and phosphocreatine.

SUMMARY

The results of study of the rapidity of metabolism of adenosinetriphosphate (ATP) and phosphocreatine (PC) in the heart in stimulation of Pavlov reinforcing nerve are presented. Experiments were performed with the aid of radioactive phosphorus both on healthy dogs and in animals with myocardial infarction. Increased heart contractions caused by this nerve in healthy dogs are associated with a more rapid metabolism of ATP and PC in the cardiac muscle. The effect of stimulation of reinforcing nerve in myocardial infarction is less pronounced. This is probably connected with the fact that the metabolism of ATP and PC in the heart is not accelerated but, on the contrary, delayed in these conditions.

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INFLUENCE OF COOLING ON THE CONTENT OF PHOSPHORUS FRACTIONS IN THE SUPERIOR CERVICAL GANGLION

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In our previous investigations [2], the effect of gangliolytic agents on the exchange of energy-rich phosphorus fractions and on the conduction of neural excitation in the superior cervical ganglion was studied. In line with the fact that hypothermia has acquired great importance in current surgical clinical practice, we dedicated ourselves to the task of studying the effect of cooling on the content of the energy-rich phosphorus fractions and on the transmission of the neural stimulus in the superior cervical ganglion.

EXPERIMENTAL METHODS

The experiments were conducted on urethane-narcotized cats weighing from 1.5 to 3.5 kg. The functional state of the ganglion was checked on the basis of the contraction of the nictitating membrane in response to stimulation of the preganglionic trunk of the sympathetic nerve by electrical current. An ASM-3 type stimulator, generating square- wave impulses, served as the source of the stimulus. The impulses had a frequency of 40 cps, the duration of each individual stimulus was 3-8 millisec, the excitation being extended over a period of 10 seconds. The contractile actions of the nictitating membrane were registered on the smoked tape of a kymograph.

Artificial hypothermia was achieved by sandwiching the animal between dry and conventional ice. The animal was placed on a double-walled examination table. The hollow formed by the table was filled with ice water. The animal's temperature was measured with a mercury thermometer placed in the rectum. The cooling varied, in different experiments, from 28 to 15 deg.

After the required cooling temperature had been reached, the superior cervical ganglion was quickly removed and immediately frozen in liquid nitrogen. In its frozen state, the ganglion was weighed on a torsion balance and then ground for 10-15 minutes with ice-chilled 5% trichloroacetic acid. The precipitate was then removed by centrifugation. The filtrate so obtained served for the determination of the phosphorus fractions. Inorganic phosphorus was precipitated by a magnesial mixture. In the centrifugate, after removal of the inorganic phosphorus, phosphocreatine was determined on the basis of inorganic phosphoric acid present, formed in acid medium in the presence of ammonium molybdate, on standing in a constant-temperature oven for 40 minutes at 37 deg. The content of adenosinetriphosphate (ATP) with its adenosinediphosphate (ADP) impurity, was taken as the difference between the phosphate content in the sample subjected to a 7-minute hydrolysis and phosphocreatine. Phosphorus was determined using the microchemical technique of Kuttner and Cohen. The experiment and control were usually carried out on the same animal, since it had been established by us earlier that the right and left ganglion of one and the same animal differed slightly in the content of the phosphorus fractions [2]. The experiments were usually performed in the following order: after suitable preparations had been made, the normal reaction of the nictitating membrane in response to irritation of the preganglionic nerve fiber of the sympathetic nerve was measured by electrical current, was measured. In some experiments, the respiration and pulse were also registered. The control ganglion was then quickly excised and frozen. The animal was then cooled

to the required temperature (28-26-20-18-15 deg), after which the experimental ganglion was taken for investigation (just as in the case of the control, respiration and pulse were registered in individual experiments).

EXPERIMENTAL RESULTS

Three series of experiments were carried out in the present work. In the first series of experiments, the influence of the temperature on the transmission of nerve excitation in the superior cervical ganglion and on the general state of the animal were studied. In the second series of experiments, the effect of cooling on the content of the phosphorus fractions present in the superior cervical ganglion was studied. In the third series of experiments, the influence of ATP on the transfer of the neural stimulus in the superior cervical ganglion under conditions of blockage of synaptic conduction was clarified through the technique of cooling.

First series of experiments. In this series of experiments, the animal was cooled to a temperature of 15-17 deg (i.e. to provide a possibility of carrying out the observations in all phases of the cooling). The beast was then warmed. The animal was sometimes recooled, after warming, to the assigned temperature (17-15 deg). The experiments demonstrated that cooling from 37-35 deg (the normal rectal temperature) to 17 deg by the method indicated could be achieved in $1\frac{1}{2}$ to 2 hours, while the recooling (after warming) took place in much less time. In 2 experiments, we had the possibility of comparing the periods required for the onset of cooling in decerebrate and narcotized cats. The onset of cooling in decerebrate cats occurred much sooner (in 1 hour to an hour and ten minutes) than was the case in narcotized cats. At the onset of cooling, i.e. at a 30-26° temperature, a stepping-up of the respiration and pulse rates was observed, while in some of the experiments, an enhancement of the tonus of the nicititating membrane in response to excitation of the preganglionic trunk of the sympathetic nerve by electrical current was noted. Further cooling to 25-23° resulted in a dropoff in the reaction of the nictitating membrane, and a slowing down of respiration and pulse. At 18-15°, the reaction of the nictitating membrane ceased entirely, breathing stopped, and then the heart followed suit. Survival of the animals without controlled respiration was then out of the question. However, after cooling to 18-15°, it was possible to restore the vital activity of the animal by warming it. The cold water in the reservoir was replaced with hot water to bring about warming. Hot water bottles were applied to the animal. In 5-10 minutes (after warming was initiated) the respiration and pulse rates increased, the reaction of the nictitating membrane improved, and, in 30-40 minutes the original level was reattained (this corresponded to a temperature of 36-34 deg). When the beast was warmed to a temperature above 40 deg, sharp dyspnea and blocking of ganglionic transmission were observed.

Second series of experiments. In this series of experiments, the animals were cooled to 28-25-22-20-18-15 deg. On cooling to 28-26 deg, most of the experiments showed an increase in ATP and phosphocreatine content, with essentially no change recorded in inorganic phosphorus content. The reaction of the nictitating membrane in response to irritation of the preganglionic trunk of the sympathetic nerve was enhanced. Cooling to below 25 deg caused a diminution of the reaction of the nictitating membrane, although ATP content retained its high levels. On cooling to below 22-20 deg, a drop in ATP and phosphocreatine content was observed, along with an increase in the content of inorganic phosphorus. The reaction of the nictitating membrane dropped sharply in the process. At a 18-15 deg temperature, the content of ATP and phosphocreatine dropped off sharply, while inorganic phosphorus increased; the reaction of the nictitating membrane disappeared entirely (cf. Table below).

Third series of experiments. In this series of experiments, a solution of ATP in a 1-2 mg/kg dose was administered via intravenous route, on a background of blockage of conduction caused by cooling down to 25-20 deg. The ATP dose was taken equal to a dosage which in our previous experiments facilitated the conduction of excitation in the superior cervical ganglion. The experiments showed that the administration of ATP, under this experimental arrangement, failed to facilitate the conduction of the neural stimulus, on the contrary hindering its transmission in a majority of the experiments.

DISCUSSION

Our data stand in agreement with the viewpoint expressed in the literature to the effect that, at different degrees of cooling, dissimilar functional states are to be observed. For example, A. A. Izbinsky, A. A. Kalikhman, J. W. Buzen [5,6,1] and others conditionally divide the duration of hypothermia into 4 phases. The first phase

Influence of Cooling on Conduction of Neural Stimulus and on Content of Phosphorus Fractions in Superior Cervical Ganglion (averaged data)

Temperature	Reaction of nictitating membrane	ATP + ADP content, as % of control	Phosphocreatine content as % of control	Content inorganic phosphorus as % of control	Breathing, % of control	Pulse rate, % of control
36°	Normal	100	100	100	100	100
28—26°	Elevated	271	122	95.5—116	160	120
22—20°	Depressed	52.7	84	155.5	60	76
18—15°	Depressed to point of block	12.4	38.2	122	25	40

involved cooling from 35 deg down to 29 deg: the second phase, cooling from 29 to 27 deg, the third, cooling from 21 to 19 deg, and the fourth, cooling to below 19 deg. All physiological functions are compensated in the first and second phases, and total decompensation of all physiological functions takes place in the third and fourth phases.

In the compensated cooling phase (i.e. the first and second phases), we observed an increase in ATP and phosphocreatine content and a facilitation of ganglionic conduction in the superior cervical ganglion. During this cooling period, according to data in the literature, there occurs an enhancement of all metabolic processes [1], excitation of the vegetative centers, facilitation of transmission in the central nervous system and in the ganglionic synapses, etc. [4,7]. It is difficult to find an explanation for the accumulation of ATP which takes place during this period in hypothermia. There are 2 possible variants: it takes place either 1) because of intensified ATP formation, or 2) because of blocking of ATP breakdown.

A. E. Gurvich and M. V. Kirzon [3], by cooling an isolated fatigued rabbit muscle, obtained a temporary intensification of individual muscle contractions. In connection with this, they advanced the suggestion that the cold brings about a delay in the breakdown of some substance, which appears upon the transfer of the stimulus from nerve to muscle. The accumulation of a store of this substance thereupon becomes a possibility. Juxtaposing our data to the results of the work performed by A. E. Gurvich and M. V. Kirzon, it may be inferred that ATP was accumulated in both investigations, possibly due to a hindrance to ATP breakdown. At a temperature of 25 deg, when a blockage of ganglionic conduction is observed and the content of phosphorus fractions remains at a high level, we suggest that the excess accumulation of ATP acts as a hindrance to synaptic conduction, since it is known that ATP, in small doses, facilitates, and in large doses hinders, ganglionic transmission of stimuli.

The third series of experiments brought confirmation to the suggestion advanced, since the administration of ATP in doses which, under normal conditions, would facilitate ganglionic transmission not only failed to facilitate it, but served as a powerful hindrance.

An explanation of the lowering of the content of the phosphorus fractions at temperatures below 20 deg presents no great difficulty, since this stage of hypothermia is characterized by a lowering of all vital functions: the mechanism governing body heat gets out of commission, as a result of which a passive drop in temperature ensues, along with inhibition of the activity of the vegetative centers, and a diminution of all forms of metabolism. It is apparent that the reduction in ATP content during this phase may be explained by an impairment in the processes involved in phosphorylation, as a consequence of the total decompensation of all vital functions.

On the basis of the experiments carried out, it may be concluded that, on cooling to 28 deg, there were observed a facilitation of ganglionic transmission and an increase in ATP and phosphocreatine content in the superior cervical ganglion. The inorganic phosphorus content remained essentially unchanged.

On cooling to 25 deg, blocking of ganglionic transmission was observed, although ATP and phosphocreatine content increased, while inorganic phosphorus either dropped or showed no change when compared to normal conditions. When ATP was administered in a 1-2 mg/kg dose, under conditions of cooling ganglionic transmission was obstructed.

When the animal was cooled to a 20-15 deg temperature, the content of the phosphorus fractions in the superior cervical ganglion showed a decrease, with ganglionic transmission being hampered, at the same time, up to the point of complete block.

SUMMARY

Experiments were performed on cats under urethane anesthesia. The effect of cooling on the content of the phosphorus fractions and transmission of nervous excitation in the superior cervical ganglion was studied. Experiments showed that ganglionic transmission is facilitated in cooling to 28°C. There is likewise increased content of adenosinetriphosphate (ATP) and phosphocreatine (PC) in the superior cervical ganglion, while the content of inorganic phosphorus remains almost the same. The ganglionic transmission is hindered in cooling to 25°C. However, the content of ATP and PC is increased in comparison with normal conditions, while the content of inorganic phosphorus is decreased or remains unchanged. The ganglionic transmission is hindered in introduction of ATP in the dose of 1 to 2 mg/kg of the body weight in conditions of cooling. Cooling of animals down to the temperature of 15 to 20°C is connected with decrease of phosphorus fractions and inhibition of ganglionic transmission up to complete block.

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PHARMACOLOGY

THE INFLUENCE OF ANTICHOLINERGIC SUBSTANCES ON THE FUNCTIONAL STATE OF THE SUPERIOR CERVICAL GANGLION

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The present communication presents a comparative evaluation of the activity of a number of anticholinergic substances with respect to their influence on the functional state of the superior cervical ganglion.

EXPERIMENTAL METHOD

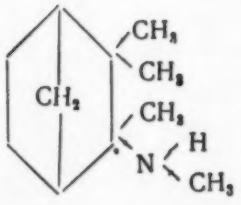
Experiments were performed on decerebrate and anesthetized (urethane) cats. Contractions of the nictitating membrane and bioelectric currents of the postganglionic fibers were recorded with various frequencies and definite duration of preganglionic trunk stimulation. The level of lability before and after administration of pharmacologically active substances was established (D.A. Kharkevich [3,4,5,6]). In control experiments the pessimal reaction usually occurred at frequencies of 60-100 cps, although sometimes inhibition was not marked even at frequencies of 150-200 cps. This is connected not only with individual variations between animals but also with the technique of the operation itself. Thus, for example, when transection of the preganglionic sympathetic trunk precedes decerebration the pessimum occurs at higher frequencies than when the sequence is reversed, and so on.

In some experiments, in addition to electric stimulation of preganglionic fibers, chemical stimulation of the ganglion was also used. This was achieved by acetylcholine which was introduced in amounts of 40-80 γ in a retrograde manner into the lingual artery. The external carotid artery was tied in these experiments.

The following anticholinergic substances were investigated: mecamine* (mecamylamine), atropine, scopolamine, d-tubocurarine and decamethonium. The substances were injected into the femoral vein.

EXPERIMENTAL RESULTS

The first of the substances investigated – mecamine – is a new long-acting ganglion – blocking agent (C. A. Stone, M. L. Torchiana, A. Navarro and K. H. Beyer [12]; A. E. Doyle, E. A. Morphy, G. H. Neilson [8]). Chemically, the preparation is 3-methylaminoisocamphane, i.e. a secondary amine (see formula):



* Mecamine was synthesized in the Chemistry Department of the Institute of Pharmacology and Chemotherapy, AMN SSSR.

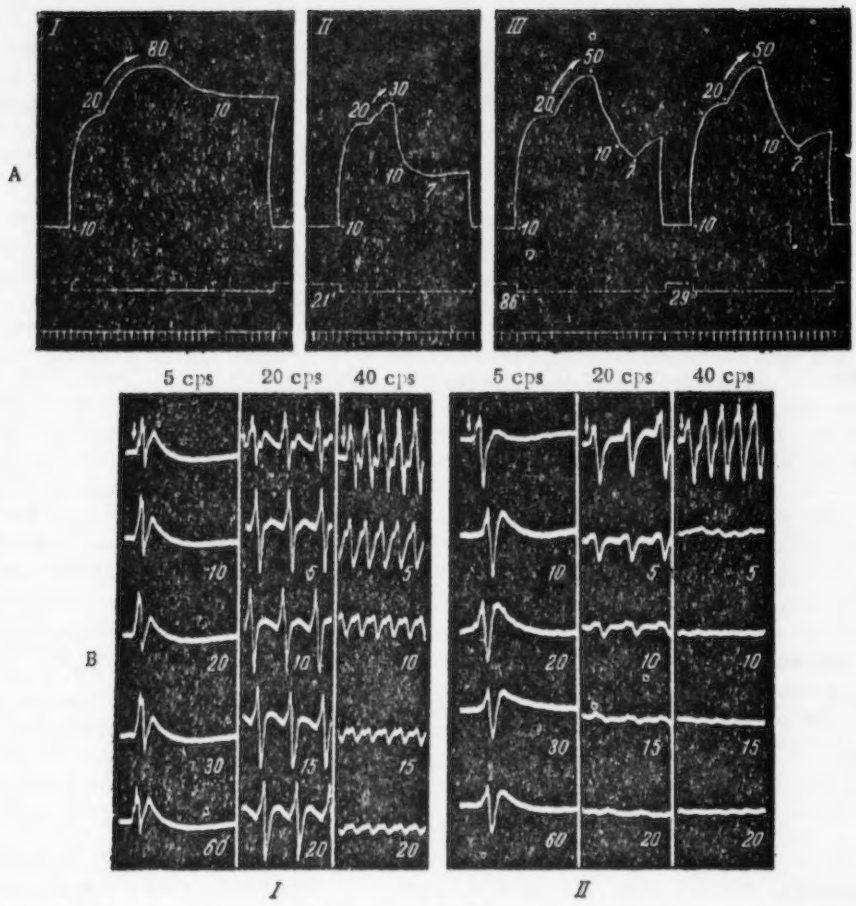


Fig. 1. The effect of mecamine on lability of the superior cervical ganglion. A) Mechanograms of nictitating membrane contractions. Figures designate frequency of stimulation of preganglionic trunk (in cps). Arrows correspond to gradual transition from one frequency to the next (I) before administration of mecamine; II) 21 minutes after injection of 0.5 mg/kg mecamine; III) 107 minutes after injection. B) Biocurrents of postganglionic fibers. Frequency of preganglionic trunk stimulation is marked over each column of oscillograms. Vertical arrow (↓) marks beginning of stimulation. Figures over each oscillogram designate time from beginning of stimulation in seconds (read from above down) (I) before mecamine injection; II) 9 minutes after injection 0.5 mg/kg mecamine). Experiments A) and B) were performed on decerebrate cats with maximal amplitude of stimulation and duration of each stimulus of 0.5 millisecond (A) and 0.1 millisecond (B).

As shown experimentally, mecamine has a profoundly depressing effect on the functional state of the superior cervical ganglion. The threshold dose for this substance is 5-10 γ/kg. In doses of 0.25-0.5 mg/kg mecamine shifts the pessimum markedly towards the lower frequencies, the duration of this effect being several hours. Thus, Fig. 1, A) demonstrates that 21 minutes after administration of 0.5 mg/kg mecamine the pessimal reaction occurred at the frequency of 30 cps, where as it was barely perceptible at 80 cps prior to injection

of the substance. It is characteristic that the change from the pessimal frequency of stimulation (30 cps) to the optimal (10 cps) was not accompanied by restoration of the level of the curve. At the frequency of 7 cps relaxation of the nictitating membrane ceased and its tonus began to increase slowly. After 2-2½ hours following injection of mecamine the pessimum occurred at 50 cps despite the fact that the initial amplitude of nictitating membrane contraction at 10 cps as well as the total height of the curves practically reached the original level. In this case too the pessimal reaction was not abolished by an abrupt change of stimulation frequency from 50 to 10 cps. However, when the frequency of stimulation was decreased to 7 cps transmission of excitation in the ganglion was restored. The nictitating membrane tonus began to increase perceptibly. The duration of the mecamine effect can also be judged from Fig. 1, A) which shows that after administration of 0.5 mg/kg mecamine the reduction of ganglionic lability continues for well over 2½ hours. According to data obtained by Yu. V. Uranov therapeutic doses (1-2.5 mg/kg) of mecamine depresses autonomic ganglia for periods of 6-18 hours.

In the dose of 1 mg/kg, mecamine causes considerable reduction in ganglionic excitability. The pessimum is shifted even more sharply towards the lower frequencies and occurs at rhythms which had been optimal prior to administration of this substance. The events are shown to be occurring in the ganglion itself by changes in the biopotentials of the postganglionic fibers. Results of one such experiment are presented in Fig. 1, B). The oscillograms show that following administration of 0.5 mg/kg mecamine the most definite changes are observed at the higher frequencies of stimulation. Thus, following administration of mecamine the biopotentials, at 40 cps, almost completely disappeared by the 5th second after beginning of stimulation. At the 15th-20th second barely perceptible oscillations were observed at 20 cps. Prior to administration of mecamine definite biocurrents were recorded at these frequencies and with analogous intervals of time; these biocurrents were synchronous with the rhythm of preganglionic trunk stimulation. It must be mentioned that at 5 cps the biocurrents were somewhat increased under the influence of mecamine.

Fig. 2 and 3 show that following administration of 1 mg/kg mecamine, the biopotentials of the postganglionic fibers at frequencies of 20-60 cps only appeared in response to the first few stimuli after which they disappeared. Considerable changes were also observed at 5 cps, i.e. at such a frequency at which, under ordinary conditions, the potentials were of high voltage and maintained their amplitude for prolonged periods of time.

Comparison of the results obtained with data published earlier concerning quaternary ammonium bases (D. A. Kharkevich [4,5,6]) leads to the conclusion that mecamine lowers the lability of the superior cervical ganglion for a substantially longer period of time than hexonium and pentamine. Mecamine approaches hexonium* in its activity.

Besides mecamine, compounds with predominant activity in the region of cholinereactive structures were investigated. The first of these – atropin – was used in doses from 0.5 to 4 mg/kg. It was found that this substance in doses of 1-3 mg/kg slightly diminished the functional lability of the ganglion (by 10-20 cps). These changes are only observed in those cases in which the excitability of the ganglion is appreciably decreased. Transmission of excitation in the superior cervical ganglion when atropine was used in the dose of 1 mg/kg diminished by an average of 15-25%. More pronounced lowering of the amplitude of nictitating membrane contractions (of the order of 50%) was noted in isolated experiments, even when the dose of the substance was increased to 2-4 mg/kg.

When the postganglionic fiber biocurrents were recorded it was found that atropine diminished the amplitude of the potentials slightly (Fig. 2).

The second preparation in the atropine group – scopolamine – proved to be an even less active compound. Maximal changes in ganglionic transmission which were expressed in diminution of the nictitating membrane reaction did not exceed 5-10%, and were, moreover, inconstant. No changes in the functional state of the superior or cervical ganglion could be detected. The doses of scopolamine used in these experiments were from 0.3 to 5 mg/kg.

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* Transliterated from Russian – probably hexamethonium.

The third group of anticholinergic substances investigated included two muscle relaxants with different mechanism of action – d-tubocurarine and decamethonium.

D-tubocurarine was used in doses 0.15-0.3 mg/kg. This preparation caused definite decrease in ganglionic lability. The experiments showed that following administration of this substance the pessimum was shifted towards the lower frequencies and relaxation of the nictitating membrane occurred more sharply than in the control experiment. The excitability of the ganglion changed only slightly (usually not more than by

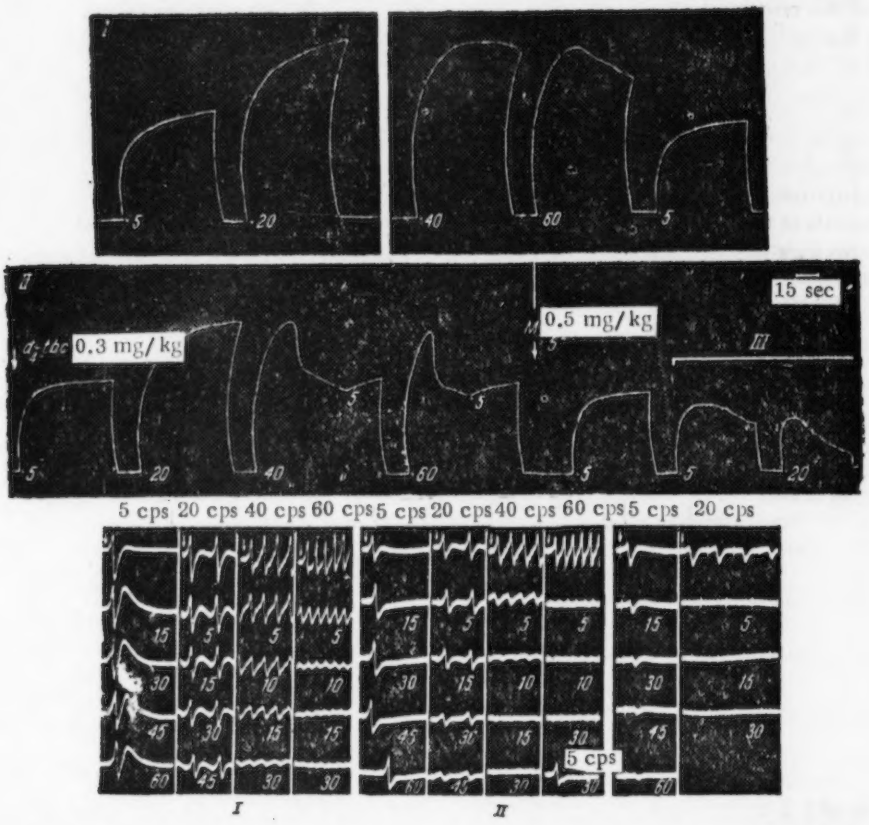

Fig. 2. Postganglionic fiber biocurrents. Vertical arrow (↓) beginning of stimulation. Figures underneath each oscillogram – time from the beginning of stimulation in seconds (read from above down). Frequency of preganglionic trunk stimulation (in cps) marked under each column of oscillograms.
I) Before administration of the substance, II) 4 minutes after injection of atropine 1 mg/kg; III) 8 minutes after injection of mecamine 1 mg/kg. Experiment performed on decerebrate cat. Duration of each stimulus 0.1 millisecond. Amplitude of stimulus – supramaximal.

10-20% at 10 cps). Duration of d-tubocurarine action in such experiments was on the average 1-1½ hours.

The inhibitory action of d-tubocurarine on the functional lability of the superior cervical ganglion was also confirmed oscillographically. The postganglionic fiber biocurrents were somewhat diminished following administration of the substance and ceased to be reproduced after a shorter interval of time than prior to its use. Some experiments were staged with simultaneous recording of biopotentials and of nictitating membrane contractions (Fig. 3). These experiments demonstrated particularly convincingly that d-tubocurarine enhances

pessimal inhibition in the ganglion. If at the moment of pessimal reaction, accompanied by absence of biopotentials and relaxation of the nictitating membrane muscle, the pessimal frequency was replaced by a known optimal (e.g. 5 cps) frequency restoration of the membrane tonus and appearance of biopotential were observed (Fig. 3, III).

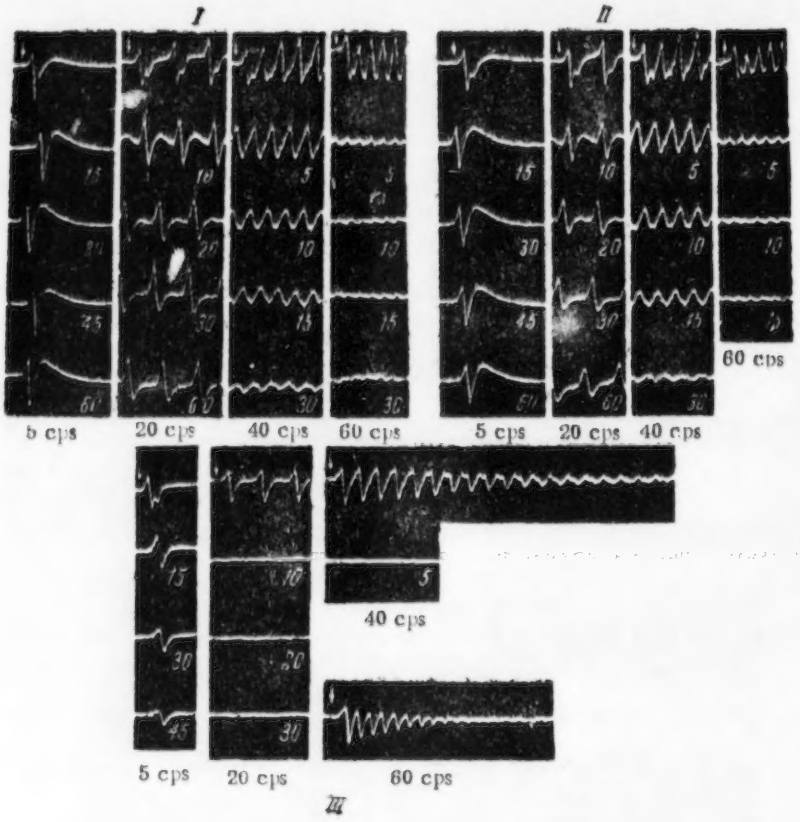

Fig. 3. Simultaneous recording of nictitating membrane contractions (A) and postganglionic fiber biopotentials (B). *
Corresponding biopotentials and mechanograms are designated by the same figures (I, II, III). Figures at the beginning of each row of mechanograms – frequency of stimulation of the preganglionic trunk (in cps). Figures underneath each oscillogram – time from the beginning of stimulation (in seconds, see from above down). Vertical arrow (↓) beginning of stimulation. I) Before administration of the substances; II) 4 minutes after injection of d-tubocurarine 0.3 mg/kg; III) 9 minutes after injection of mecamine 1 mg/kg. The experiment was performed on decerebrate cat with artificial respiration. Duration of each stimulus 0.1 millisecond. Amplitude of stimulation – maximal.

D-tubocurarine thus possesses the ability to diminish the functional lability of the superior cervical ganglion. This effect is sufficiently clearcut but considerably less pronounced than that of mecamine, hexonium or pentamine.

* A and B not identified in figure – Publisher.

Unlike d-tubocurarine, decamethonium proved to be little active with respect to the superior cervical ganglion. Changes in the height of the mechanograms following the use of decamethonium in doses from 30 to 100 γ/kg. were negligible. The ganglion lability also remained unchanged. In order to make sure that the substance reached the ganglion, the animal was given tetraethylammonium at the end of the experiment. This was accompanied by consistent lowering of the amplitude of nictitating membrane contractions, together with a shift of the pessimum towards the lower frequencies. Decamethonium actively inhibited neuromuscular transmission in doses considerably smaller than those which were used in experiments on the superior cervical ganglion.

Decamethonium can thus be taken to be without effect on the functional state of the superior cervical ganglion cells.

SUMMARY

The investigations showed that the substances tested exert an inhibitory influence on the functional state of the ganglion. The most active compound was mecamine. It lowered ganglionic lability very markedly and for a prolonged period of time. D-tubocurarine in doses which inhibit neuromuscular transmission possesses analogous but considerably less pronounced action. Decamethonium is practically without effect on the functional lability of the cervical ganglion. In this respect it resembles ditilin• which is also practically devoid of gangliоplegic activity. It can thus be taken that curare-like substances with a depolarizing type of action do not exert a negative effect on transmission of impulses in the superior cervical ganglion. In this they differ substantially from muscle relaxants with a competitive type of action (d-tubocurarine, diplacin••).

Atropine slightly lowers the functional activity of the ganglion and only with decrease in its excitability which is also slight. Scopolamine affects ganglionic transmission of impulses to an even lesser extent. Practically, these changes in ganglionic transmission can be considered as negligible. The lability of the ganglion is unaffected by scopolamine.

The present investigation and investigations published earlier (D. A. Kharkevich [3,5,6], A. V. Valdman, Z. N. Ivanova and D. A. Kharkevich [1]) have been concerned with the study of the effect of many anticholinergic substances on the functional state of the superior cervical ganglion. These have included gangliоplegic agents (mecamine, hexonium, pentamine tetraethylammonium, nicotine, pachycarpine,• sparteine), muscle relaxants (d-tubocurarine, diplacin, decamethonium, ditilin) as well as substance which are mainly active in the region of cholinereactive structures (atropine, scopolamine, spasmolytin•). Comparison of the data obtained leads to the conclusion that all the substances which inhibit transmission of impulses in the ganglion lower its functional activity. Restoration of these two parameters occurs in the reverse order. These relationships are the rule in the case of gangliоplegic substances. For some substances the influence on lability can be considered as the main manifestation of their action on the ganglion (e.g. for diplacin).

The mechanism of pessimal inhibition and its shift, under the influence of ganglion blocking agents, towards the lower frequencies still remains uncertain. Many authors (A. G. Ginetsinsky and I. M. Shamarina [2]; G. L. Brown and W. Feldberg [7] and others) consider that acetylcholine plays the chief role in the development of the pessimum. However, in recent years H. Kewitz [9,10,11] has published work in which he denies altogether participation of acetylcholine in the transmission of nervous excitation in the ganglion. This is based on the results of experiments on the perfused superior cervical ganglion of cat. The author established that atropine, scopolamine, novocain and trasentine abolished the excitatory action of acetylcholine on the ganglion without affecting the transmission of excitation associated with electric stimulation of preganglionic sympathetic fibers.

In our investigation analogous experiments were performed but on the intact animal with preservation of blood supply to the superior cervical ganglion. Acetylcholine was given by retrograde injection into the

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• Transliteration of Russian – Publisher's note.

•• Russian trade name.

lingual artery. It was found that atropine and novocain reduced the ganglionic reaction to acetylcholine and at the same time inhibited synaptic transmission in the ganglion associated with electric stimulation of preganglionic fibers. Scopolamine was without effect in either case.

Thus in our view there is yet no sufficient basis for denying the participation of acetylcholine both in the transmission of nervous excitation and the development of pessimal inhibition.

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THE INFLUENCE OF SOME DERIVATIVES OF THE PHENOTHIAZINE GROUP ON REFLEXES FROM THE HEART

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A number of investigators have established that certain derivatives of the phenothiazine group possess the ability to dilate coronary blood vessels [1,5]. Moreover, it is known that neuroplegic compounds have found application in cardiac surgery. In connection with this it appeared interesting to investigate the influence of phenothiazine derivatives on reflex reactions arising on constriction of coronary vessels. Two preparations were selected for this investigation, viz. aminazine* and mepazine**.

EXPERIMENTAL METHODS

Experiments were performed on cats under urethane anesthesia and on decerebrate cats. The effect of mepazine and aminazine on reflex changes in blood pressure and respiration upon clamping of the descending branch of the left coronary artery was studied. When the vessel was completely compressed for 10 seconds acute ischemia of the corresponding area of the myocardium occurred and was accompanied by reflex changes in blood pressure and respiration. In a series of short-term experiments and one of prolonged experiments the electrocardiographic changes associated with these phenomena were recorded; these changes were characteristic of impaired coronary circulation. The preparations being tested were given intravenously in the short-term experiments and intraperitoneally in the prolonged ones. A more detailed description of the experimental methods has already been published in an earlier communication [4].

EXPERIMENTAL RESULTS

The experiments showed that compression of the coronary artery is associated with the appearance of reflexes affecting blood pressure and respiration. In the majority of the experiments the reflexes from the heart were depressor in character (95-97%) and only in some cases were these reflexes pressor (3-5%). Reflexes affecting respiration led to increased depth and frequency of respiratory movements.

The investigation showed that aminazine in doses of 0.1-0.5 mg/kg depressed the reflexes from the heart on blood pressure by 50-67% over 7-25 minutes. When the doses are increased to 1-2 mg/kg the vascular reflexes are depressed by 70-80% for 30-40 minutes (Fig. 1, I). The maximum effect following intravenous

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* Transliteration of Russian – Publisher's note.

** Mepazine (N-methyl-piperidyl-3-methylphenothiazine) corresponds in its chemical structure to pacatal**; it was synthesized at the Chemistry Department of the Institute of Pharmacology and Chemotherapy, AMN SSSR, by S. V. Zhuravlev, A. N. Gritsenko and M. I. Dorokhova. Yu. I. Vikhlyaev has carried out a detailed pharmacologic investigation of mepazine [2].

administration of aminazine occurs after 4-7 minutes. These data were obtained in experiments on anesthetized and decerebrate animals.

In a number of experiments aminazine altered the character of the reflexes, transforming them from depressor into pressor ones.

Injection of aminazine also diminishes the reflexes affecting respiration. The experiments also showed that aminazine diminished slightly the electrocardiogram changes arising upon impairment of coronary circulation. The electrocardiogram changes associated with impaired blood supply to the heart usually consist of the following features: a sharp increase in the T-wave, inversion of the ventricular QRS complex, deviation of the ST interval from the isoelectric line, disturbance of rhythm (Fig. 1, II). Such electrocardiographic changes, arising on compression of the coronary artery, are not abolished but only somewhat diminished by intraperitoneal injection of 1 mg/kg aminazine. The effect lasts for 25-30 minutes.

It must be noted that aminazine possesses, in addition to its depressing effect on reflexes from the heart, marked hypotensive properties. In connection with this it seemed essential to find out the relationship between changes in reflexes from the heart and the level of blood pressure. Artificial lowering of blood pressure was achieved in 2 ways: 1) acute blood-letting and 2) intravenous injection of 1-2 mg/kg papaverine*.

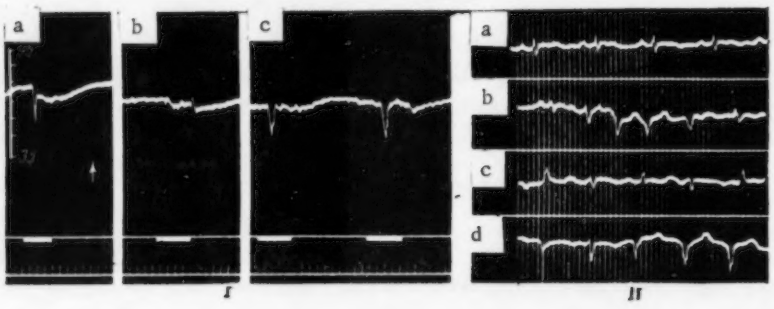

Fig. 1. Effect of aminazine on reflexes from the heart. Cat weighing 2.5 kg. Decerebrate.
I) Change in reflexes affecting blood pressure under the influence of aminazine: a) background; b) 10 minutes after intravenous injection of 2 mg/kg aminazine; c) 31 and 34 minutes after injection of aminazine. Records (from above down): blood pressure, stimulus mark, time marker (5 seconds).
II) Changes in the electrocardiogram on clamping the descending branch of the left coronary artery: a) normal cat electrocardiogram; b) electrocardiogram with the coronary artery clamped; c) electrocardiogram 10 minutes after intravenous injection of aminazine 1 mg/kg; d) electrocardiogram 30 minutes after injection of aminazine.

It was established on the basis of these experiments that as the blood pressure decreased, the reflexes from the heart showed some diminution. When the blood pressure is lowered by 10 mm Hg the reflexes from the heart decrease by 10-20% (the variability of the changes is connected with the initial absolute magnitude of the reflex). Following injection of aminazine under conditions of lowered blood pressure to a similar level, the vascular reflexes diminish by 50-60%. These data permit the conclusion that aminazine possesses a marked ability to inhibit reflexes from the heart.

* Control experiments showed that papaverine did not depress the reflexes from the heart.

The experiments also showed that aminazine inhibited the reflex changes in blood pressure and respiration for considerable periods of time. This raised the question whether the magnitude and character of the reflexes did not alter on compression of the coronary artery for prolonged periods of time (3-4 hours) without the administration of any pharmacologic substances. It was demonstrated that such was not the case.

Mepazine, in doses of 1-2 mg/kg, diminishes the reflexes from the heart by 35-50% over 20-30 minutes (Fig. 2, I). These data were obtained on anesthetized animals and animals decrebrated at the collicular level.

It must be emphasized that mepazine, which exerts a markedly weaker effect on the reflexes from the heart than aminazine, is substantially more effective than the latter in normalizing the electrocardiographic changes associated with impaired nutrition of the heart muscle. Thus, intraperitoneal and intravenous injections of 2-5 mg/kg mepazine abolish completely the electrocardiographic changes for 1-2 hours (Fig. 2, II). Normalization of the electrocardiogram under the influence of mepazine consists of the following: diminution of the T-wave, disappearance of inverted QRS complexes, increase in voltage of the waves, restoration of regular rhythm. These data showed that mepazine improves cardiac blood supply under conditions of myocardial ischemia produced by clamping the coronary artery.

Since many of these experiments were performed on anesthetized animals it seemed interesting to discover the effect of narcotic substances on the effect of neuroplegic compounds relative to reflexes from the heart. There are references in the literature [3] indicating that narcotic substances (urethane, medinal) exert a weak effect on reflexes from internal organs. A special series of experiments was therefore performed on animals immobilized by means of the curare-like preparation ditilin•. It was found that aminazine given to

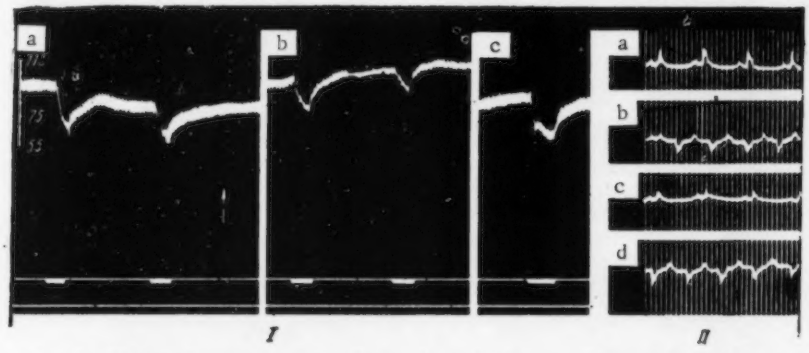

Fig. 2. Effect of mepazine on reflexes from the heart. Cat weighing 4 kg. Urethane anesthesia.
I) changes in blood pressure reflexes under the influence of mepazine: a) background; b) 4 and 5 minutes after intravenous injection of 1 mg/kg mepazine; c) 25 minutes after injection of mepazine. II) Electrocardiogram changes upon clamping of the descending branch of the left coronary artery: a) normal cat electrocardiogram; b) electrocardiogram upon compression of the coronary artery; c) electrocardiogram 15 minutes after intravenous injection of 1 mg/kg mepazine; d) electrocardiogram 1 hour after injection of mepazine.

ditilin-immobilized cats in the dose of 1 mg/kg diminished reflexes from the heart by 55-60% for 25-30 minutes. Mepazine under analogous conditions (2 mg/kg) inhibited reflex changes in blood pressure by 30-40% for 15-25 minutes.

• Ditilin was given intravenously in divided doses (150-200 mg in 20 ml solution) throughout the experiment.

The experiments thus showed that aminazine and mepazine inhibited reflexes from the heart to a somewhat lesser extent in unanesthetized animals. Evidently, the effect of the neuroplegic substances under investigation is enhanced to some extent in the presence of anesthesia. However, no substantial differences could be obtained under different experimental conditions.

Taking into account the long duration and complexity of surgical operations on intrathoracic organs, it is extremely important to be able to diminish maximally reflexes from the heart by means of pharmacologic agents, at the same time maintaining for as long as possible normal blood supply to the heart muscle.

It has already been indicated that mepazine abolishes completely disturbances of blood supply to the myocardium with preservation of reflexes from the heart which are a little diminished. At the same time, it is well known that many neuroplegic compounds are able to potentiate the action of analgesics.

It was shown earlier that analgesics (phenadon*, promedol*, morphine and tecodin*) inhibited reflex changes of blood pressure and respiration associated with compression of the coronary artery [4]; the most active substances as regards reflexes from the heart were phenadon and promedol.

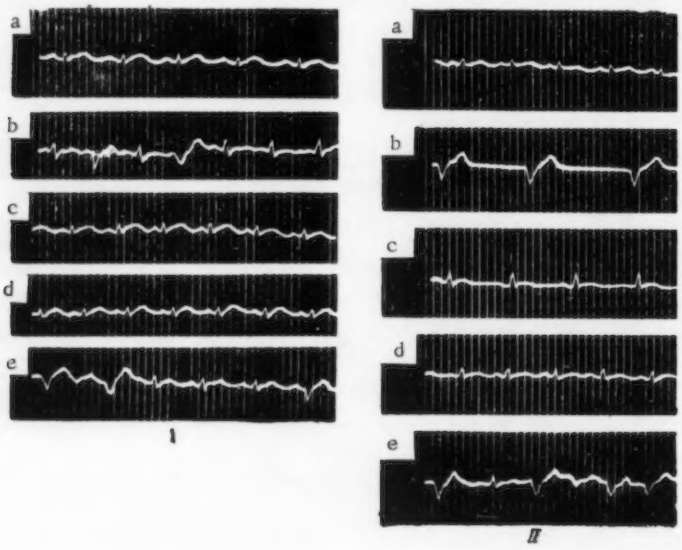

Fig. 3. Changes in the electrocardiogram upon compression of the descending branch of the left coronary artery. Cat weighing 4.4 kg (long-term experiment).
I, a) Electrocardiogram of cat prior to experiment; b) electrocardiogram with compression of coronary artery; c) electrocardiogram 30 minutes after intraperitoneal injection of 5 mg/kg mepazine and 0.25 mg/kg phenadon; d) electrocardiogram 24 hours after injection of mepazine and phenadon; e) electrocardiogram 50 hours after injection of mepazine and phenadon. II, a) normal cat electrocardiogram; b) electrocardiogram with compression of coronary artery; c) electrocardiogram 30 minutes after intraperitoneal injections of 5 mg/kg mepazine and 1 mg/kg promedol; d) electrocardiogram 3 hours after injection of mepazine and promedol; e) electrocardiogram 4 hours after injection of mepazine and promedol.

As has been mentioned above, mepazine has more marked capacity for improving myocardial blood supply than aminazine. Observations mentioned above formed the basis for a special series of experiments on the combined action of analgesics and mepazine. The experiments were carried out according to the following scheme. Under long-term conditions, the unanesthetized animal was given 0.25 mg/kg phenadon intraperitoneally and 20 minutes later 5 mg/kg mepazine by the same route. In a number of experiments the two preparations were given simultaneously. It was found that mepazine and phenadon, when used in combination in the doses indicated, abolished completely the electrocardiographic changes arising upon compression of the coronary artery. The effect produced by these preparations lasted 48-50 hours (Fig. 3, I). The combined action of mepazine and promedol was investigated in a similar manner. The dose of promedol used was 1 mg/kg, of mepazine 5 mg/kg. These two substances used in combination smooth out the electrocardiographic changes over a period of 4 hours (Fig. 3, II). These data indicate that the combined administration of analgesics and mepazine prolongs the action of the preparations to a considerable extent (5-20 fold).

The present investigations permit the conclusion that mepazine and aminazine promote diminution of reflex changes of blood pressure and respiration arising upon occlusion of the coronary arteries. The data obtained also demonstrate that aminazine exerts a stronger effect on reflexes from the heart than mepazine. However, mepazine abolishes completely the electrocardiographic changes associated with impairment of cardiac blood supply. Aminazine lacks this property almost entirely.

The present experiments suggest that the combined use of mepazine and analgesic substances may prove useful in clinical work.

SUMMARY

Short-term experiments were performed on anesthetized and decerebrate cats. A comparative study was carried out on the effect of aminazine and mepazine on the reflex reactions when cardiac blood supply was impaired.

It was established that mepazine and aminazine depressed the reflexes from the heart and decreased the electrocardiographic changes. The effect of aminazine on the reflexes from the heart is more pronounced than that of mepazine.

The latter completely eliminates the changes in the electrocardiogram connected with disturbance of coronary circulation. In the case of aminazine this property is almost absent. In combined administration of mepazine and analgesic substances (phenadon, promedol) the effect of these preparations is considerably prolonged.

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THE EFFECT OF ANTICHOLINESTERASE PREPARATIONS ON THE DARK ADAPTATION OF THE EYE

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The present work is a continuation of investigations carried out in this department on the study of the effect of anticholinesterase preparations on varous functions of the body [6,7,8 and 9]. Its object was to investigate the effect of eserine and prostigmin on the dark adaptation of the eye in the healthy human subject.

EXPERIMENTAL METHOD

After a 30 minute period of dark adaptation in a dark room, a 5 minute exposure to light was given. Thereafter, every 5 minutes, the light sensitivity of areas of the retina situated within 15° of the macula was measured with a Nagel adaptometer during adaptation of the eye to darkness.

The light sensitivity of the eye was determined by a Kravkov-Vishnevsky apparatus and a model "AM" Belostotsky-Hoffmann adaptometer in accordance with its operational instructions.

For each subject we determined the rate of dark adaptation and the light sensitivity of the eye before and after the intramuscular injection of physiological saline (0.6 ml) and after intramuscular injection of the preparations. The following preparations were used: eserine (Physostigminum salicylicum [Merck] in a dose of 0.2-0.6 mg, prostigmin (Prostigminum [La Roche]) and proserine* (Proserinum, [NIKhFI]), in doses of 0.15-0.3 mg. To determine the duration of the action of the anticholinesterase preparations, the dark adaptation and light sensitivity of the eye were examined for 2 days.

EXPERIMENTAL RESULTS

The rate of adaptation, measured by Nagel's apparatus, is shown from results of 41 observations (Tables 1 and 2).

In the normal subject with no preparation injected or after injection of physiological saline, dark adaptation of the eye was effected in 25-50 minutes, being complete on the average in 41 minutes. All the experiments of injection of physiological saline gave the same results as without injection. Injection of all the anticholinesterase preparations gave acceleration of adaptation by 2-5 times (average 3) in all experiments without exception.

In the normal subject, without injection and after injection of physiological saline, the threshold of dark

* Prostigmin and proserine are identical preparations (the methyl sulfate of the dimethylcarbamino complex ether of oxyphenyltrimethylammonium).

adaptation of the eye averaged 2400-4000 Nagel adaptometer units, with a mean value of 3200. Injections of anticholinesterase preparations increased the sensitivity of the eye by $1\frac{1}{2}$ - 2 times, with a mean of $1\frac{1}{2}$ times. It was shown by special observations that the action of prostigmin (in a dose of 0.3 mg) lasted 37 hours in subject I. N., after which the rate of dark adaptation and the level of light sensitivity were restored to their original values.

TABLE I

Rate of Dark Adaptation by Nagel's Method in Several Experiments

Subject	O.M.	I.N.	A.D.	A.S.	N.Zh.	Arithmetic mean
Age	46 years	19 years	19 years	26 years	19 years	
With no preparation injection	35. 35. 35. 40	50. 50	35	30	25	41
After injection: of physiological saline	—	50. 50. 50	35. 35. 35. 35. 40	25. 30	25. 25	41
of eserine	15. 20	10. 10	15. 15. 15	10. 10	10. 10	13.7
of prostigmine	—	10	15. 15	10	—	13.7
of proserine	—	10	20. 20	15. 15	—	13.7
Coefficient of acceleration	2.2—2.0	5	2.3—2.0	3—2	2.5	3

The light sensitivity of the eye was determined in 24 subjects (44 investigations) with the Kravkov-Vishnevsky apparatus and in 44 subjects (50 investigations) with the Belostotsky-Hoffmann adaptometer.

The curves in Fig. 1 demonstrate the changes in the light sensitivity of vision in 24 subjects after intramuscular injection of physiological saline and eserine (taken from mean values). The abscissa represents time after injection in minutes and the ordinate shows the time in seconds during which the subject distinguished the object placed in the apparatus. After injection of eserine the time of distinguishing the object was shortened from 37.8 to 12.4 seconds, i. e. threefold. In some experiments it was reduced to 2-3 seconds.

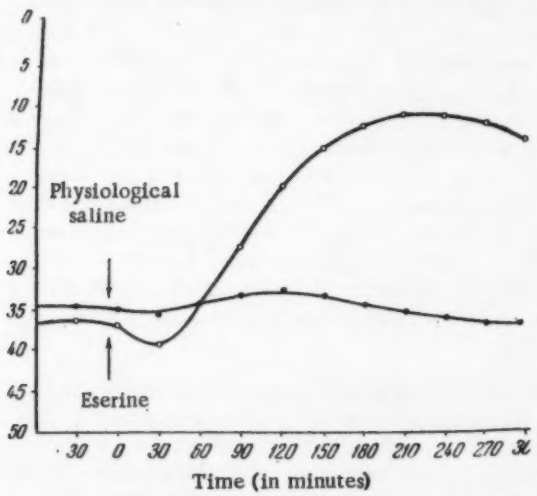

Fig. 1. Effect of eserine on light sensitivity of the normal eye as determined by the Kravkov-Vishnevsky apparatus (mean of 44 observations on 24 subjects).

It must be emphasized that the doses of anticholinesterase preparations which we injected, while increasing the achromatic vision and corresponding to 30-40% of the therapeutic dose given in the Pharmacopeia of the USSR, did not produce any vegetative changes nor influence the subjective state of the experimental subjects.

Investigations with the Belostotsky-Hoffmann adaptometer also showed acceleration of the dark adaptation process and increase in light sensitivity as a result of eserine. The level of dark adaptation corresponding to 40th minute in control experiments was observed at the 20th minute after injection of eserine. The initial light sensitivity (at the 4th minute) was increased on the average from 520 to 1250 relative units to from 30,000 to 54,000 units on the 20th minute and from 68,000 to 110,000 units after 40 minutes. The mean values of the experimental results from 44 subjects are shown in Fig. 2.

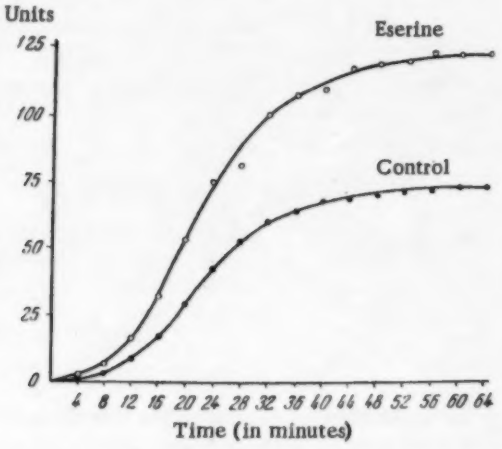

Fig. 2. Effect of eserine on dark adaptation of the normal eye as determined by the Belostotsky-Hoffmann Adaptometer (mean of 50 observations on 44 subjects).

Thus the anticholinesterase preparations described, considerably accelerate dark adaptation (from 2-5 times) and increase by $1\frac{1}{2}$ times the sensitivity of achromatic vision. This testifies to the importance not only of synthesis of visual purple but also of synaptic acetylcholine in the production of dark adaptation to a definite sensitivity of achromatic vision. According to investigations by W. Feldberg [10], the retina contains a large amount of an enzyme synthesizing acetylcholine, while this enzyme is absent from the optic nerve trunk. Bunin [1] showed that in dark adapted retinas of frogs there is a larger acetylcholine content than in light adapted. It must be admitted that perception of light by the subject at the time of dark adaptation depends not only on the state of the acetylcholine mediator of the retina, consisting of a three-membered neuronal chain, but also on the synapses of the external geniculate body and the projection zones of the cerebral cortex, i. e. on mediator exchange in all the links of the optic analysor. A single injection of strychnine in experiments by Kravkov [3,4] also invariably increased the power of discrimination of the eye on the 2nd-3rd day. This effect of strychnine, similar to the action of eserine and proserine, is understandable considering its anticholinesterase properties [11,12,13].

The action of eserine and prostigmin on spinal reflexes, as was first shown by Schweitzer, Stedman and Wright [14] is variable: eserine increases the excitability of the knee reflex, its power and amplitude, while prostigmin shows an inhibitory action on this reflex. In our investigations [8], we demonstrated the great stability of this stimulatory action (not changing into a depressing action) on the motor neuron cell and the respiratory center of the frog, of even massive doses of eserine (100-200 mg/kg), at the same time the biphasic character of the action of considerably smaller doses of proserine (2-10 mg/kg) on the same systems of the frog was shown. The first phase of excitation from the action of proserine usually changed rapidly into a prolonged and stable phase of parabiotic inhibition. Similar differences in the action of eserine and proserine were also noted by us [9] in relation to spinal reflexes in the dog.

TABLE 2

Changes in the Dark Adaptation Threshold (in Nagel Test Units) after Injection of Anticholinesterase Compounds.

Subject	O. M.	I.N.	A.D.	A.S.	N.Zh.	Arithmetic mean
Age	46 years	19 years	19 years	26 years	19 years	
No preparation injected	3000 3000 2400 2800	3500 3500	3000	3000	4000	3220
After injection of physiological saline	—	3500 3500	3000 3000 3000 3000 3000	3000 3000	4000 4000	
Eserine	1400 2000	2500 2200	2500 2600 2500	2000 2000	2000 2000	2150
Prostigmin	—	2000 2000	2400 2000	2000	—	
Proserine	—	2000	2300 2500	2000 2000	—	
Coefficient of increase of sensitivity of the eye		1.6	1.6	1.5	2	1.5

The present investigation shows, however, that eserine and proserine act equally and monophasically on the optic analysor. Raev [5] showed the same thing by investigation of the rheobase and chronaxie of the eye. Eserine and proserine lowered the rheobase by 20-25% and lengthened the chronaxie (phosphene) 2.0-6.6 times by comparison with their original state.

The equal – in the type of stable catelectrotonus – action of eserine and proserine on the optic analysor and the different action on the spinal motor neurons of animals and the respiratory center of the frong are the result of the unequal level of lability and functional stability of these particular nerve structures. By the action of eserine and proserine on the foveal sensitivity of the eye, Kravkov [4] and Zaretskaya [2], show that proserine increases the sensitivity of the eye to the whole visible spectrum, while small doses of eserine increase the sensitivity to red and large doses to green light.

SUMMARY

The effect of eserine (Physostigmum salicylicum, Merck) and prostigmin or proserine on the dark adaptation and sensitivity of the eyes to light was studied in healthy adult individuals. Investigations were carried out with the

adaptometer of Nagel, the adaptometer of Belostotsky-Hoffman, as well as with the aid of Kravkov-Vishnevsky apparatus. Intramuscular introduction of small doses of eserine solution (from 0.2 to 0.6 mg) or prostigmin (from 0.15 to 0.3 mg) accelerated the dark adaptation of the eyes and increased the sensitivity of the eye by 1.5-3 times. This effect of eserine and prostigmin is stable and remains for 24 hours or even longer. It is monophasic stimulatory in type. The authors explain the above effect of various anticholinesterase preparations by increased mobilization of the synaptic acetylcholine in the retina, the genu of the internal capsulae and the corresponding projection zone of the brain cortex.

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ONCOLOGY

THE ANTIGENS OF THE CANCER CELL

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Research directed to the study of the antigens of malignant tumors has in the past occupied the attention of many oncologists. In work by L. A. Zilber and his co-workers [1], and also in research by P. N. Kosyakov [2], A. K. Saakov [3] and other writers, it was shown that in addition to specific cancer antigens the tumor contains a large number of proteins, similar or identical in their antigenic properties to normal tissue proteins.

In the present work we set out to investigate the presence of species, organ and cancer antigens directly in the cells of a malignant tumor, using for this purpose a suitable type of experimental cancer.

EXPERIMENTAL METHOD

The investigation was done on an ascitic variant of the Ehrlich mouse adenocarcinoma. Analysis of the antigenic properties of the tumor was performed by the anaphylaxis with desensitization method. In the experiments we used guinea pigs weighing 250-300 g. The guinea pigs were sensitized subcutaneously to the tumor by a dose of 15 mg. Desensitization and the determining injections of antigens in corresponding doses were performed intracardially 3-4 weeks after the first protein injection.

EXPERIMENTAL RESULTS

The use of an ascitic form of mouse cancer in our investigation had definite advantages since it permitted the analysis of antigens directly entering into the composition of the cancer cells. Malignant cells floating freely in the ascitic fluid could easily be freed from foreign protein by repeated washing with physiological saline, thus preventing it from taking part in the anaphylactic reactions. We assessed the degree of freedom from foreign protein of the cancer cells by the anaphylactogenic properties of the water used for washing. Preliminary experiments showed that washing the cells seven times produced maximum freedom from foreign proteins.

As may be seen from Table 1, guinea pigs sensitized by washing fluid in a volume of 0.05 ml•, showed no reaction on injection of 0.5-1 ml of original antigen.

The guinea pigs showed no reaction to the injection of 0.3 ml of mouse serum. However, subsequent injection into these guinea pigs of a saline extract of washed cancer cells produced clear anaphylactic shock in all the animals used in the experiment.

• A dose of 0.05 ml exceeds by 8 times the quantity of fluid present in one sensitizing dose of cancer cell precipitate.

TABLE 1

Antigenic Properties of the Supernatant Fluid obtained by Washing Ehrlich Mouse Adenocarcinoma Cells 7 Times

Serial No.	Guinea pig No.	Sensitization	Desensitization									
		7th wash adenocarc	7th wash adenocarc cells		Mouse serum						Mouse adenocarcinoma	
		Dose (ml)	Dose (ml)	Reaction	Dose (ml)	Reaction	Dose (ml)	Reaction	Dose (ml)	Reaction	Dose (mg)	Reaction
1	2985	0.05	0.5	—	0.3	—	·	·	·	·	50	++
2	3354	0.05	1.0	—	0.3	—	·	·	·	·	50	+++
3	2591	0.05	1.0	—	0.3	—	·	·	·	·	50	+
4	3853	1.00	1.0	—	0.3	++	0.3	++	0.3	—	50	+
5	3920	1.00	1.0	±	0.3	+	0.3	+	0.3	—	50	+++
6	3958	1.00	1.0	±	0.3	+	0.3	—	0.3	—	50	+++
7	3789	1.00	1.0	—	0.3	++	0.3	++	0.3	—	50	++

Conventional signs: – no reaction; + brief nose scratching with the paw; ++ stronger nose scratching, sneezing, shortness of breath, bristling of the fur, cough; +++ the same but more pronounced, passage of urine and feces; ++++ convulsions, leaping about, spasms, terminating usually in death of the animal; · no injection given.

A similar pattern could be seen in guinea pigs sensitized to washing fluid injected in a volume of 1 ml. In this case the guinea pigs remained insensitive to the original antigen but showed a feeble reaction to mouse serum and reacted to a greater degree to injection of tumor extract.

Thus the experimental results show that repeated washing of cells of ascitic cancer with physiological saline largely frees the tumor cells from foreign protein, in particular from proteins which are similar to the serum proteins in their antigenic structure, i. e. to the specific proteins of normal tissues. The experiments also showed that the washings contained a considerable quantity of protein of related origin to intracellular tumor proteins. We may suppose that during washing of the cancer cells there is a gradual removal of normal proteins and a parallel accumulation of tumor proteins which is possibly due to destruction of some of the tumor cells during washing.

The possibility of freeing tumor cells from foreign proteins and, consequently from risk of causing anaphylactic reactions, enabled us later on to make a series of experiments to analyze the antigenic properties of the proteins directly entering into the composition of malignant cells. We were particularly interested in the question of whether the proteins of the malignant cell possess any species antigenic specificity. For this purpose we sensitized a group of guinea pigs by subcutaneous injections of 15 mg doses of washed cancer cell precipitate. As antigen, to test the specific properties of the cancer cell protein, we used mouse serum.

The experiments showed (Table 2) that mouse serum, injected intracardially into the guinea pigs, caused an anaphylactic reaction in all the animals. A selected dose of 0.5 ml of serum proved adequate to desensitize the animals to serum protein on the first injection, since on repeating the injection of serum the guinea pigs showed no reaction. As we might have expected, desensitization of the guinea pigs to serum protein did not remove their sensitivity to subsequent injection of the original antigen. After injection of extract of cancer cells, 2 of the 4 animals used in the experiment died from anaphylaxis and the remaining two survived severe anaphylactic shock.

Guinea pigs in the second group, sensitized to mouse cancer cells but not desensitized to mouse serum, reacted violently to injection of cancer cell extract so that all the animals died as a result from anaphylactic shock. Unsensitized guinea pigs did not react to injection of these particular antigens (control of the toxicity of the antigen).

The results of this experiment show that proteins which in their antigenic properties are close to the proteins of mouse serum enter into the composition of the cancer cells. In other words, the intracellular protein of a mouse tumor possesses the species properties of the normal tissue proteins.

TABLE 2

Species Antigen in Ehrlich Adenocarcinoma Cells.

Serial No.	Guinea pig No.	Sensitization	Desensitization				Determining injection	
		Mouse adenocarc.	Mouse serum				Mouse adenocarc.	
		Dose (mg)	Dose (ml)	Reaction	Dose (ml)	Reaction	Dose (mg)	Reaction
Experiment								
5	2700	15	0,5	++++	0.5	—	50	++++
6	1103	15	0.3	+++	0.5	±	50	++
7	1350	15	0.5	+++	0.5	—	50	Fatal shock
8	3333	15	0.5	+++	0.5	—	50	The same
Control of sensitization								
1	1793	15	.	.	.	.	50	" "
2	2865	15	.	.	.	.	50	" "
3	1232	15	.	.	.	.	50	" "
4	2116	15	.	.	.	.	50	" "
Control								
9	3032	.	0.5	—	0.5	—	50	—
10	1773	.	0.5	—	0.5	—	50	—
11	2119	.	0.5	—	0.5	—	50	—

Conventional signs the same as in Table 1.

The fact that desensitization of guinea pigs to species protein does not remove, but only to some extent weakens, their sensitivity to subsequent injection of the original protein is evidence of the antigenic complexity of the cancer cell.

Bearing in mind that the tumor serving as the object of our investigation is related in origin to the spontaneous breast cancer of mice, we may suppose that besides the species antigen, this tumor includes in its composition protein which, in its antigenic properties, is identical or close to the organ antigen of mouse breast cancer. In order to test this hypothesis we made experiments in which as a desensitizing agent we used mouse breast tissue.

As also in the previous experiment, the guinea pigs were sensitized to cells of the mouse tumor. Desensitization was first brought about by mouse serum and later by an extract of mouse breast. As a determining injection the guinea pigs received the original antigen. The experiments showed (Table 3) that guinea pigs, fully desensitized to serum protein, clearly reacted by anaphylactic shock to the injection of an extract of mouse breast. However, after full desensitization to this antigen also, they retained their sensitivity to the original antigen. On injection of extract of cancer cells, all the animals in the experimental group developed severe anaphylactic shock. Controls, in the form of unsensitized animals, showed no reaction whatever on injection of these antigens.

Analysis of the antigenic properties of the tumor by the use of breast tissue therefore showed that among antigens taking part in the composition of the cancer cell are some which are similar in their properties to breast antigen. It is possible that in the cancer cell may be found proteins which, in their antigenic properties, are similar to proteins of other organs and tissues of mice.

These facts are evidence that in a cancer cell are found proteins, similar or identical in their antigenic properties to proteins of mouse serum and breast. In addition, as the experiments showed, along with species

TABLE 3

The Presence of Organ Antigen in Ehrlich Adenocarcinoma Cells

Serial No.	Guinea pig No.	Sensitization: Mouse adenocarc.	Desensitization: Mouse serum											Desensitization: Mouse breast				Determining injection: Mouse adenocarc.	
		Dose (mg)	Dose (ml)	Reaction	Dose (ml)	Reaction	Dose (ml)	Reaction		Dose (ml)	Reaction	Dose (ml)	Reaction	Dose (mg)	Reaction	Dose (mg)	Reaction	Dose (mg)	Reaction
Experiment																			
1	2189	15	0.3	++++	0.5	±	0.5	—	Interval 20 hours	0.5	++++	0.5	++	30	+++	30	±	50	++++
2	2775	15	0.3	++++	0.5	+	0.5	—		0.5	—	.	.	30	++	30	±	50	++++
3	2200	15	0.3	++++	0.5	±	0.5	—		0.5	—	.	.	30	+++	30	±	50	++++
4	1989	15	0.3	+++	0.5	+	0.5	—		0.5	++	0.5	—	30	++	30	±	50	Fatal shock
Control																			
5	3071	.	0.3	—	0.5	—	0.5	—		0.5	—	0.5	—	30	—	30	±	50	—
6	3391	.	0.3	—	0.5	—	0.5	—		0.5	—	0.5	—	30	—	30	—	50	—
7	3118	.	0.3	—	0.5	—	0.5	—		0.5	—	0.5	—	30	—	30	—	50	—

Conventional signs the same as in Table 1.

and organ antigens the tumor contains a whole complex of other proteins, among which are those which are and those which are not found in normal organs and tissues, the latter being regarded as specific tumor proteins.

SUMMARY

Ascitic variation of mice adenocarcinoma served as an object of investigation. Analysis of antigenic properties of the tumor was conducted by the method of anaphylaxis with desensitization on guinea pigs. Repeated washing of floating cells of mice cancer from foreign proteins gave the opportunity to investigate the antigens which constitute the content of the tumor cells. The presence of proteins which are similar or identical to the proteins of the serum and the mammary glands was demonstrated in cancer cells. Besides species-specific and organ-specific antigens, numerous other antigens are found in cancer cells, among which the specific tumor antigen is evidently present.

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EXPERIMENTAL BIOLOGY

A COMPARATIVE STUDY OF HUMORAL AND CERTAIN MORPHOLOGICAL CHANGES RESULTING FROM HOMOTRANSPLANTATION OF SKIN

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At the present time little doubt remains about the problem of the immunobiological basis of death of the homograft. There is a great deal of evidence that cellular factors of the recipient play a leading part in tissue incompatibility (N. N. Elansky [3]; I. Khutnaya [5]; Darcy [8]; Simonsen, Buemann, Hammeltoft and others [13].

There is also more evidence in the literature that antibodies appear in the serum of the recipient after homotransplantation (E. A. Zotikov [4]; D. Amos and others [6]; P. Gorer [9]; E. Muirhead and M. Groves [11]).

However the problem of the relation of these antibodies to the survival or death of the homograft has not yet been thoroughly studied.

In this work we set out to investigate whether these antibodies take part in the creation of tissue incompatibility. For this purpose, along with a study of the serological properties of the recipients' sera, we made a capillaroscopic investigation in vivo of the transplanted skin grafts.

There are descriptions in the literature of the vascular changes occurring in transplanted skin grafts (I. Lehrfeld and A. Taylor [10]; R. Scothorne and I. McGregor[12]; LConverse and F. Rapoport [7] and others).

These authors point out that at first in the transplanted skin graft there occurs restoration of the circulation by anastomosis formation between the vessels of the recipient and those of the graft. Later on stasis and thrombosis develop in the homografts, accompanied by destruction of the grafts themselves. This work is purely morphological in character and is not concerned with changes brought about by the humoral factors of the recipient.

For our research we made use of the ordinary capillariscope or the MBS-2 binocular loupe system. Capillaroscopic examinations were undertaken from the 2nd-4th day after grafting, at intervals of 1-2 days, until either final cessation or complete survival of the circulation of the graft. On capillaroscopic examination of 4 cases, owing to impossibility of adequately illuminating the skin to see the superficial vascular network of the graft, we scarified the epidermis slightly with a razor. If circulation was present in the graft it rapidly filled with blood.

For serological examination we used the ordinary hemagglutination reaction in tubes.

EXPERIMENTAL METHOD AND RESULTS

Experiments were performed on 35 rabbits weighing 1750-3500 g, mainly of the chinchilla variety, which were grafted with full thickness skin flaps measuring 50-100 cm^2. Homografting was used with 22 animals, autografting in 13 animals and in 6 rabbits treated by autografting, homografts also were applied a few month after-

wards. All the skin homografts died in the first 2 weeks after grafting. The autografts survived and were kept under observation for more than 6 months.

Observations on the process of survival of skin grafts showed that the vascular changes arising at first in auto- and homografts are very similar. For the first 2-3 days after operation, the grafts were lacking in circulation and were pale in color. The vessels were dilated and had lost their clear outlines. Pink areas appeared in the grafts 3-4 days after operation. Capillaroscopy revealed that the dilated vessels were full of stationary blood (Fig. 1). In places in the vessels of the auto- and homografts slow movement of blood could already be seen. Evidently at this time, vessels had grown in the receptor area and had begun to form anastomoses with vessels in the graft. After 5-6 days the whole graft had become rose pink or red in color. The vessels were filled with blood. Movement of blood could be seen in them.

Beginning on the 6th day after transplantation, disturbances of the circulation in the homografts began to appear. The color of the graft changed in 24 hours from pink or red to dark blue or even black. The blood flow ceased. On capillaroscopy hemorrhages were seen along the course of the vessels (Fig. 1,b). Evidently in the vessels of the homograft, thrombosis has begun to occur, accompanied by rupture of the walls of the vessels or disturbances of their permeability leading to the formation of multiple hemorrhages.

Further changes in the homografts comprised drying and conversion into a scab, under which old blood gathered and epithelialization of the wound proceeded. At the end of the first or middle of the second month after transplantation, the wound margins had drawn together, and a scar had formed in place of the skin defect. In two rabbits (Nos. 786 and 77) the process of survival and death of the homografts proceeded somewhat differently. On the development of hyperemia in the vessels of the homografts, thrombosis did not develop. Their color became less clear. Simultaneously on the 10-12th day after transplantation, the epithelia of the homografts became intensely desquamating. The grafts became covered with scabs consisting of desquamating scales. When the scabs were removed at first, the surface of the graft beneath was pink in color, but later ulceration of the epithelium was observed. The vessels of the homograft could not then be distinguished. In the two cases described we regarded this as the moment of death of the homografts. Later, the homografts dried and were converted into scabs.

A comparative analysis made with autotransplantation of skin grafts shows that after 6-9 days their color was pale. The vessels acquired a clearer outline (see Fig. 1). From 10-12 hours after transplantation the autografts can hardly be distinguished from the surrounding skin. Sometimes slight desquamation of the epithelium from the grafts is observed.

Serological examination of the recipient rabbits with homografts showed that in 19 out of 28 of them antibodies were formed. In one recipient not showing the presence of antibodies, on examination of the serum by means of the ordinary hemagglutination reaction it was possible to detect incomplete antibodies. For the examination of this serum for incomplete or monovalent antibodies a standard weight of erythrocytes was prepared not in physiological saline but in a 20% solution of human serum albumin.

In none of the 13 rabbits with autotransplanted skin grafts were antibodies found.

Comparing the changes in antibody development with the character of the vascular changes arising in the skin grafts, a correlation is shown between the antibodies appearing in the serum of the recipient and disturbances of the circulation in the homograft. By way of illustration we quote data for rabbit recipient No. 1236 (Figure 2).

Until the 7th day after homoplastic grafting the serum of the recipient contained no antibodies. Commencing on the 5th day there were hemorrhages in the graft. On the 7th day after grafting, antibodies were found in a titer of 1 : 4. Disturbance of the circulation in the homograft corresponded to the time of discovery of antibodies, showing itself on the 7th day after operation also.

Similar results were obtained on examination of the majority of the other rabbit recipients. These results are given in the table.

From the results given it is clear that the disturbance of circulation or the start of death of the homografts occurs as a rule on the 6th-9th day after transplantation, and in the majority of cases this corresponds

Fig. 1. Progress of the vascular changes in auto- and homotransplanted skin grafts in rabbits. a) vessels in the graft 3-4 days after transplantation. The capillaries are dilated and filled with blood. In places there is slow movement of blood; b) vessels in homograft 6-9 days after transplantation. Hemorrhages are seen along the course of the vessels; c) vessels in the autograft 6-9 days after transplantation. The capillaries are filled with flowing blood.

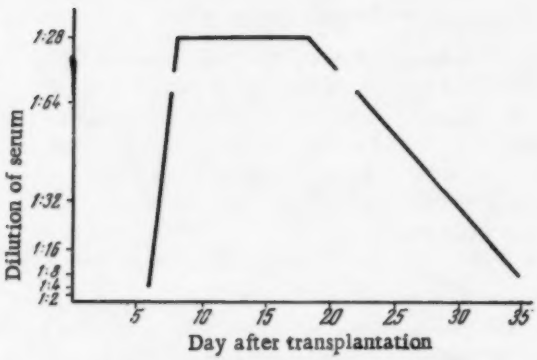

Fig. 2. Changes in the antibody titer.

to the time of discovery or of increase in the amount of antibodies in the serum of the recipient. Only in two recipient rabbits Nos. 786 and 77 were antibodies found on the 5th-9th day after transplantation, while death of the homograft ensued at the end of the second week. The cause of death of the homografts in rabbits Nos. 786 and 77 is not clear. It may be mentioned, however, that in relation to the immune serum of various recipient rabbits, the erythrocytes of recipient rabbits Nos. 786 and 77 were very similar to the erythrocytes of their donors.

Thus in the serum of the majority of recipient rabbits, immune antibodies appeared as a result of homoplastic skin grafting. In 8 recipients, no antibody could be detected by the method which we were using. It is possible that it was present in small quantity or in a state of incomplete antibody.

In support of this idea may be quoted the results of a number of authorities who found that incomplete antibodies appeared in the blood of mice and dogs after homoplastic grafting of skin and kidney (Muirhead and Groves). The absence of antibody production in response to the homografted tissue could also be a result of the lowered immunological reactivity of the rabbits to produce antibodies. We must bear in mind that tissue incompatibility, like any other immunological condition, is determined not only by humoral but also by cellular factors.

From the results mentioned above it is clear that survival of auto- and homografts of skin in the first few days after transplantation was very similar. Antibodies appeared in response to homografted tissue and not to autografted tissue, and moreover the onset of death of the homografts corresponded in time to the discovery of antibodies. This all suggests that antibodies are the response of the recipient to the antigenically foreign tissues of the donor. In work on immunological relationships, M. R. Gashek observed during successful homografting of skin between partners in embryonic parabiosis, loss of

Morphological and Humoral Changes arising with Homoplastic Skin Grafting in Rabbits

Identification No. of Recipient Rabbit / Identification No. of Donor Rabbit	Appearance of hemagglutinins – recipient serum (days after transplant)	Disturbance of circulation signs of homograft death (days after transplant)
1236/1717	6	6
71/811	7	7
812/813	5	6
177/829	7	7
510/46	6	7
811/71	7	6
818/407	9	Between 5th and 9th
77/14	9	11—15
786/407	5	9—15
442/407	Between 7th and 8th	Between 7th and 8th
407/818	Before 8th	7
1717/1236	Between 9th and 12th	6
292/603	6	7
643/701	Between 8th and 18th	8
701/643	Before 14th	8
365/333	6	Before 10th
333/365	5	Before 10th
756/786	6	9
866/67	Between 8th and 9th	Between 9th and 10th
67/670	Antibodies not found	7
936/71	» » »	7
14/77	» » »	7
46/813	» » »	7
813/46	» » »	8
603/292	» » »	Between 8th and 9th
829/177	» » »	Between 7th and 8th
235/333	» » »	6
363/786	» » »	9

the power of the recipient to produce antibodies (hemagglutinins) against the donor's erythrocytes.

In addition the extensive experience of bacteriology and also noninfective immunology, in particular blood transfusion, which is essentially a homotransplantation of fluid tissue, shows that antibodies arising in the body in response to the introduction of a foreign antigen always lead to destruction or rapid removal of the antigen.

This may serve only to prove that antibodies arising as a result of homotransplantation of skin are also an indication of the reaction of the recipient directed towards removal of the foreign antigen and preservation of the normal internal environment of the body.

SUMMARY

Antibodies were found in 19 out of 28 rabbit recipients which were examined following homoplastic transplantation of the skin. There were no antibodies found in 13 rabbits with autotransplanted flaps of skin. With the aid of capillaroscopy it was established that disturbance of circulation in the homotransplantate corresponded to the time when the antibodies were found in the serum of the recipient.

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THE STUDY OF THE ANTIGENIC PROPERTIES OF TISSUES AND ORGANS OF ANIMALS IN ONTOGENESIS

COMMUNICATION V. THE STAGE-SPECIFIC ANTIGENS OF THE CRYSTALLINE LENS

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In previous communications we discussed the question of the appearance of species and organ antigenic specificity of the crystalline lens of the duck and of the heart of the chick [4,5,6,7]. Undoubtedly the complex structural changes undergone by organs in the process of development are closely related to metabolic changes and consequently, to changes of the protein biosynthesis type. Changes of metabolism and of protein biosynthesis in their turn must lead to changes in the antigenic properties of certain organs. From this starting point we set out to answer the problem of whether there are specific antigens for each stage of development of the crystalline lens.

There are reports in the literature suggesting that in the embryonic period of development, the serum [2,11,17,19], erythrocytes [12,14,15,16,20], muscular tissue [9,10,18] and other organs [1] of man and various animals contain proteins (antigens) which differ from the corresponding proteins (antigens) of the adult.

We were able to find only one work in which the writers demonstrated the presence in the crystalline lens of the chick of antigens specific for the definite stages of development. V. Burke, N. Sullivan, H. Petersen and R. Weed [13] report that antigens of the crystalline lens of chick embryos 96 hours after incubation react with anti-crystalline lens serum of embryos 160 hours after incubation but do not react with such serum 300 hours after incubation, whereas, antigens of the crystalline lens of embryos 120 hours after incubation react with anti-lens serum of embryos 300 hours after incubation but not with anti-lens serum of adult chicks. Unfortunately the writers did not make a detailed study of the morphogenesis of the crystalline lens, and therefore on the basis of their data, it is difficult to connect the presence of certain antigens at definite stages of development with any of the morphological features which are characteristic of the crystalline lens at these stages. As we know, the crystalline lenses of 300 hour chick embryos and those of mature chicks are very similar in structure. The question of why the crystalline lenses of 120 hour embryos reacted with anti-lens sera of 300 hour embryos, but not with anti-lens sera of mature chicks, remains unsolved.

The present investigation was undertaken with the object of further clarification of this problem.

EXPERIMENTAL METHODS

In this work we used the reaction of anaphylaxis with desensitization [3]. The experiment was set up as follows. Guinea pigs (15 in number) were sensitized with suspensions of crystalline lens tissue of 90, 130, 192, 264 and 360 hour duck embryos. On the 21st day after the sensitizing injections the animals were desensitized to species and organ specific antigens (2 intraperitoneal injections of extract from the crystalline lens of an adult duck). Two hours after the second desensitizing injection a test of the completeness of de-

sensitization was carried out. Two hours after this, the animals received intravenously a determining injection of antigen. For the determining injections we used the same antigens which were used for sensitizing the animals.

EXPERIMENTAL RESULTS

The experimental results are shown in Table 1. As can be seen from this Table, in all the sensitized animals in response to the injection of extract • from crystalline lens tissue of an adult duck, there was observed a marked positive reaction; some of these animals even died from anaphylactic shock. On testing the completeness of desensitization, no positive anaphylactic reaction was detected. The animals which were sensitized to the lens of 90 and 130 hour embryos gave a positive reaction, assessed as "++", in response to the determining injections. Guinea pigs which were sensitized to suspensions of lens tissue of 192, 264 and 360 hour duck embryos did not give a positive reaction in response to the determining injection of antigen.

Thus the experimental results given in Table 1 show that in the crystalline lens tissue of 90 and 130 hour embryos besides the species and organ specific antigens which are common to the lens of the adult duck, there are also found antigens, specific for these stages of development.

However, giving our attention to the results of previous experiments [4] in which it was shown that the antigenic species specificity of the crystalline lens was very prominent in early stages of its development, we became doubtful of the completeness of desensitization to species specific antigen of the guinea pigs which had been sensitized to the lenses of 90 and 130 hour embryos. To settle this point we made the following experiment.

Guinea pigs (9 in nuber) were sensitized to suspensions of crystalline lens tissue of 130, 192 and 360 hour embryos. On the 21st day after the sensitizing injections, all the animals were desensitized to species and organ specific antigens (two subcutaneous injections of extract of lens tissue from adult ducks the day before carrying out the reaction). On the morning of the day the reaction was to be performed, tests were made of the completeness of desensitization to organ specific antigen (intravenous injection of extract of lens tissue of an adult duck). If the guinea pigs did not show a positive reaction, then 2 hours later tests of completeness of desensitization to species specific antigen were made by the intravenous injection of adult duck serum. Two hours after these tests the animals received intravenously the determining injection of antigen. For this purpose we used the same antigens which had been used to sensitize the animals.

The result of this experiment are shown in Table 2, from which it can be seen that the test of completeness of desensitization to species specific antigen did not give a positive reaction.

Guinea pigs sensitized to crystalline lens tissue of 130 hour embryos gave a positive reaction, classed as "+", in response to the determining dose of antigen. At the same time, the animals sensitized to lens tissue of 192 and 360 hour embryos showed no positive reaction in response to the determining injection of antigen. Two unsensitized guinea pigs were injected intravenously with extract of lens tissue of 130 hour duck embryos (technical control), and these did not show a positive reaction.

The results of this experiment confirm the findings of the previous experiment on the presence in tissue of the crystalline lens of embryos of an early stage of development (90 and 130 hours incubation) of antigens specific for these stages. We called these antigens "stage specific" [8].

The morphological investigation which was performed showed that at this period of development (90-130 hours) intensive growth of the primary lens fibers was observed, determining the high rate of growth of the entire crystalline lens (see Figure)••. Estimation of ribonucleic acid (RNA) by Brashe's method showed also

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• The preparation of the extract was described previously by us [4].

•• In constructing this graph we used the results of calculations of the volume of the lens in embryos of various stages of incubation (3-4 embryos on each day). The volume of the lens was determined in histological specimens by the formula for the volume of a revolving ellipsoid.

TABLE 1

Anaphylaxis Reaction in Guinea Pigs Sensitized with Suspensions of Duck Embryo Crystalline Lens Tissue in Response to Injection of Lens Tissue Extract from Fully Grown Ducks and Embryos

Sensitization (subcutaneously)		Desensitization •(intraperitoneally)			Test complete of desensit (intravenously)			Determining injection (intravenously)		
Antigen	Dose	Antigen	Dose (mg)	Reaction	Antigen	Dose (mg)	Reaction	Antigen	Dose (mg)	Reaction
Suspension of crystalline lens tissue of 90 hour duck embryos	80 crystalline lenses	Extract lens tissue fully grown ducks	500	±	Extract lens tissue fully grown ducks	400	—	Extract lens tissue 90 hr. duck embryos	200[2•]	++
The same	The same	The same	500	±	The same	400	—	The same	200[3•]	+
» »	» »	» »	500	±	» »	400	—	» »	200[4•]	++
Suspension of crystalline lens tissue of 130 hour duck embryos	1.6 mg	» »	500	++	» »	400	—			
								Extract lens tissue 130 hr. duck embryos	200	++
The same	1.6 »	» »	500	++	» »	400	—	The same	200	+
» »	1.6 »	» »	500	++	» »	400	—	» »	200	+
Suspension of crystalline lens tissue of 192 hour duck embryos	1.6 »	» »	500	+++	» »	400	—	Extract lens tissue 192 hr. duck embryos	200	—
The same	1.6 »	» »	500	++	» »	400	—	The same	200	—
» »	1.6 »	» »	500	++++						

• Desensitization was performed fractionally: 2 injections of 250 mg after 6 hours (some animals died from anaphylactic shock from the first injection).

2•,3•,4• In view of the great difficulty of extracting and determining the exact weight of the crystalline lenses in 90 hour embryos, in the preparation of 200 mg of extract we took not 20 mg of tissue as usually, but 300 lenses.

Note. ++++ death of the animal from anaphylactic shock; +++ severe shock but the animal survived; ++ well marked shock; + slight shock; ± clear signs of shock absent; — total absence of any signs of shock.

TABLE 1 (Continued)

Anaphylaxis Reaction in Guinea Pigs Sensitized with Suspensions of Duck Embryo Crystalline Lens Tissue in Response to Injection of Lens Tissue Extract from Fully Grown Ducks and Embryos

Sensitization (subcutaneously)		Desensitization (intraperitoneally)			Test complete of desensit. (intravenously)			Determining injection (intravenously)		
Antigen	Dose	Antigen	Dose (mg)	Reaction	Antigen	Dose (mg)	Reaction	Antigen	Dose (mg)	Reaction
Suspension of crystalline lens tissue of 264 hr. duck embryos	1.6 мг	Extract lens tissue fully grown ducks	500	++++						
The same	1.6 »	The same	500	+++	Extract lens tissue fully grown ducks	400	—	Extract lens tissue 264 hr. duck embryos	200	—
» »	1.6 »	» »	500	++	The same	400	—	The same	200	—
Suspension of crystalline lens tissue of 360 hr. duck embryos	1.6 »	» »	500	++	» »	400	—	Extract lens tissue 360 hr. duck embryos	200	—
The same	1.6 »	» »	500	+++	» »	400	—	The same	200	—
» »	1.6 »	» »	500	++	» »	400	—	» »	200	—
Suspension of crystalline lens tissue of fully grown ducks	1.6 »	» »	500	+++	» »	400	—	Extract lens tissue fully grown ducks	200	—
The same	1.6 »	» »	500	+++	» »	400	—	The same	200	—
» »	1.6 »	» »	500	+++	» »	400	—	» »	200	—

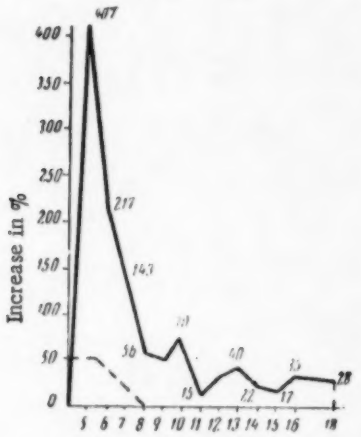
TABLE 2

Anaphylaxis Reaction in Guinea Pigs Sensitized with Suspensions of Duck Embryo Crystalline Lens Tissue in Response to Injection of Extract from Duck Embryo Crystalline lens Tissue

Sensitization (subcutaneously)		Desensitization •			Test of completeness of desensitization (intravenously) to antigen						Determining injection (intravenously)		
					Organic specific			Species specific					
Antigen	Dose (mg)	Antigen	Dose (mg)	Reaction	Antigen	Dose (mg)	Reaction	Antigen	Dose (mg)	Reaction	Antigen	Dose (mg)	Reaction
Suspension of crystalline lens tissue of 130 hour duck embryos	1.6	Extract lens tissue fully grown ducks	500	++	Extract lens tissue fully grown ducks	300	—	Adult duck serum	250	—	Extract lens tissue 130 hr. duck embryos	200	+
The same	1.6	The same	500	++	The same	300	—	The same	250	—	The same	200	+
» »	1.6	» »	500	++	» »	300	—	» »	250	—	» »	200	+
Suspension of crystalline lens tissue duck embryos	1.6	» »	500	++	» »	300	—	» »	250	—	Extract lens tissue 192 hr. duck embryos	200	—
The same	1.6	» »	500	++	» »	300	—	» »	250	—	The same	200	—
» »	1.6	» »	500	++	» »	300	—	» »	250	—	» »	200	—
Suspension of crystalline lens tissue of 360 hour duck embryos	1.6	» »	500	++	» »	300	—	» »	250	—	Extract lens tissue 360 hr. duck embryos	200	—
The same	1.6	» »	500	++	» »	300	—	» »	250	—	The same	200	—
» »	1.6	» »	500	++	» »	300	—	» »	250	—	» »	200	—
Sensitization not carried out					Technical control						Extract lens tissue 130 hr. duck embryos	200	—
The same					The same						The same	200	—

• To prevent death of the animals the injections were given subcutaneously (250 mg morning and evening) the day before injection of the determining dose of antigen.

that in the period of intensive growth of the lens its RNA content increased in concentration. It is interesting that stage specific antigens are found in lens tissue of embryos of just this period of incubation. On the 8th day of incubation, when stage specific antigens can no longer be detected, the process of formation of secondary lens fibers begins from the lens epithelium. The primary lens fibers at this time begin to undergo conversion into the "nucleus" of the lens. The RNA content of the lens tissue, and also its rate of growth are sharply reduced on the 8th day of incubation. It may be that the presence of stage specific antigens in the lens tissue of 90 and 130 hour embryos is connected with the above mentioned morphological features of the developing lens in this period of embryogenesis.



Rate of growth of the crystalline lens of the duck with days of incubation.

The findings reported in this article do not confirm the results of the experiments of Burke and his co-workers [13] mentioned above. Our experimental results showed that stage specific antigens, by contrast with the findings of Burke and his co-workers, are present in lens tissue only during early stages of development. Thus the findings of the present work show that in the developing lens tissue of the duck in early stages of embryogenesis (90 - 130 hours) antigens are present which are specific only for these stages of development – stage specific antigens. At this period the formation and intensive growth of the primary lens fibers occurs and an increased concentration of RNA is characteristic of the lens tissue.

SUMMARY

The antigenic properties of the tissue of developing crystalline lens of ducks was studied with the aid of anaphylactic reaction with desensitization.

Morphogenesis, the rate of growth of crystalline lens and content of ribonucleic acid (RNA) in it were investigated simultaneously. It was demonstrated that during the early stages of embryogenesis (90-130 hours) the antigens are present in the tissue of developing crystalline lens, which are specific only for the definite stage of development (stage-specific antigens). There is formation and intensive growth of the primary fibers of the crystalline lens and increased concentration of the RNA during this period.

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THE IMPORTANCE OF NUCLEIC ACID AND CARBOHYDRATE METABOLISM IN CELL DIVISION

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Reports in the literature on the importance of metabolic processes in cell division are very limited; in fact there are only a few histochemical investigations of variation in the nucleic acids of the dividing cell and some data on the importance of carbohydrate metabolism in this process. Several writers [1,2,3,6 and others] have shown that during division regular changes are observed in the content of ribonucleic (RNA) and desoxyribonucleic (DNA) acids in the cell. These investigations suggest that during mitosis there are definite changes in nucleic acid metabolism of the cell. However, from our point of view they do not provide adequate grounds for a conclusion on the importance of nuclein metabolism in cell division. Changes in nucleic acids at the time of mitosis may only accompany cell division and not be a fundamental biochemical process taking part in mitosis.

To investigate this problem we set up two series of experiments in which nucleic acid metabolism was disturbed and the mitotic activity of different organs was studied. The first series of experiment involved the use of 2,4 – dinitrophenol. This compound inhibits the formation of high-energy phosphate compounds of the nucleic acid, ATP, and phosphocreatine type, concerned in respiration [4,24]. Investigations on eggs of the sea-urchin showed that dinitrophenol blocked the entry of labelled phosphorus into the nucleic acid molecule.

EXPERIMENTAL METHODS

Our experiments were performed on the cornea of white mice. Mitotic activity was determined in the cornea of the left and right eye, in each case in an area of 3.3 mm^2. In both corneas the number of dividing cells and the ratio of the two first phases of mitosis to the next two phases was calculated (phase coefficient). Dinitrophenol drops were introduced into the right eye and physiological saline into the left eye. Comparing the number of mitoses in the cornea of the left and right eyes, we could judge changes in mitotic activity due to disturbance of nucleic acid phosphorylation. This experiment with dinitrophenol was preceded by a control group of observations in which the mitotic activity of the left and right eye of normal mice was determined. As these observations showed (10 experiments), the mean variation between the number of mitoses in the cornea of the left and right eye was 9%. Dinitrophenol was introduced in a concentration of 1 : 1000 1 hour and 1 hour 30 minutes before the animals were killed. The experimental and control animals were killed simultaneously at 17 hours.

EXPERIMENTAL RESULTS

In comparing the mitotic activity of the left (control) and right (experimental) corneas, attention is drawn first to the sharp increase in the phase coefficient. This is due to a considerable increase in the number of prophases and suggests inhibition of mitosis at this phase of division. On the other hand, a reduction in the total number of dividing cells was noted, by 31% on the average (Table 1). The experimental results are quite straightforward; they show that interference with intake of phosphorus into the nucleic acid molecule leads to reduction in the number of dividing cells and inhibition of mitosis at the prophase.

TABLE 1

Change in Mitotic Activity due to Dinitrophenol

Experiment No.	Time when animals killed	Number of mitoses and phase coefficient				Relation number of mitoses the same experiment to control (%)
		Right eye (experiment)		Left eye (control)		
200	After 1 hour 30 minutes	121	9.1	230	1.0	—48
201	The same	316	5.0	431	1.3	—26.7
202	» »	279	8.2	404	1.2	—31.1
203	» »	459	1.9	355	1.5	+24
204	» »	256	3.9	396	1.2	—36
207	After 1 hour	333	8.5	520	3.5	—35
208	The same	326	7.3	453	2.2	—28
209	» »	296	25.7	497	7,1	—40.4
210	» »	372	17.5	700	10.4	—46.8
211	» »	531	5.9	724	2.7	—26.7
	Mean	329	9.3	471	3.2	—31.5

In a second series of experiments we used adenine. This compound, like purine bases in general, sharply inhibits the activity of desoxyribonuclease [5]. Adenine sulphate was injected intraperitoneally into white mice 12 hours before they were killed. Control animals were injected with a corresponding volume of physiological saline. The mitotic activity was determined in preparations of the complete cornea in an area of 1.65 mm^2. The experimental results (Table 2) show that by injection of 4-6 mg of adenine a small rise in mitotic activity is observed in the retina. On injection of larger doses of the preparation (7-8 mg) a sharp reduction in mitotic activity takes a place in the corneal epithelium – by 18 times. Thus, blocking of desoxyribonuclease leads to delay in the onset of cell division (see Table 2).

In a third series of experiments we used acriflavine hydrochloride. This compound interferes with synthesis of nucleic acids, forming insoluble complexes with them and with nucleotides. Acriflavine hydrochloride was injected subcutaneously into the back of the mouse in a dose of 1.3 mg 3 hours before killing. The mitotic activity was determined in the corneal epithelium, the intestine and the tongue (Table 3).

TABLE 2

Change in Mitotic Activity in the Cornea due to Adenine •

Dose of adenine (mg)	Number of animals	Number of mitoses	Phase coefficient
(control)	13	365.0±36.0	1.35
4—6	20	433.2±45.8	1.47
7—8	12	20.2±6.1	6.86

• In contrast to the other experimental series the animals were killed at 10 a.m.

As can be seen from Table 3, disturbance of nucleic acid metabolism by acriflavine hydrochloride leads to considerable reduction in mitotic activity in all the situations studied. Some retardation of cell division at the prophase stage is noted, judging by the rise in the phase coefficient.

TABLE 3

The Effect of Acriflavine Hydrochloride on Cell Division

Group of animals	Number animals	Number mitoses and phase coefficient					
		Cornea		Intestine		Tongue	
Control	9	118.4±21	1.3	342.2±13.4	3.0	29.0±4.8	4.8
Experiment, after introduction of acriflavine hydrochloride	12	35.6±8	5.5	64.2±13.4	6.0	2.0±1.4	2.9

The second group of experiments in this series – using the local action of acriflavine hydrochloride – is still more significant. Acriflavine hydrochloride in a concentration of 1 : 100 was instilled on the cornea of the left eye of a white mouse (11 experiments) while drops of physiological saline were introduced into the right eye.

On comparing mitotic activity in the right and left corneas a reduction in the number of dividing cells on the average of 47% compared with the control group, can be seen. At the same time there is a marked increase in the phase coefficient (from 1.3 in the control group to 5.5 in the experimental). These changes in the coefficient were due to a considerable increase in the number of prophases and suggested retardation of mitosis at this stage of division.

All the experimental series show that the nucleic acid metabolism of the cell is one of the principal biochemical processes concerned in cell division. Interference with nucleic acid metabolism leads to almost complete disappearance of mitotic activity or to retardation of mitosis at the prophase stage. These findings enable us to support the view that the synthesis of thymonucleic acid in the dividing cell occurs not during the anaphase [15,25] but during the prophase [1,3,6,18,19,26].

Naturally, the processes of nucleic acid metabolism are not the only biochemical mechanisms concerned with cell division. Several observations have led to the suggestion that carbohydrate metabolism plays an important part in the mitotic process. These observations are mainly by Bullough and his co-workers [8,9,10,11, 12,13,14,15,16]. For several years these workers have asserted that changes in carbohydrate metabolism are the principal biochemical processes which determine the onset of cell division. In experiments with starch, insulin, glucose, azides, malonates, etc, these workers show considerable changes in mitotic activity. These findings, however, are disputed by Laws [21], whose experiments did not confirm the original findings of Bullough.

In order to study the importance of carbohydrate metabolism in the process of cell division we set up several experimental series with white mice and rats. We studied cell division in the cornea, tongue and ear. We judged mitotic activity by the number of dividing cells in a constant area (1.65 mm^2) and by the phase coefficient. Dividing cells were counted on 4 cuts, each 10 μ wide, taken every 100 μ from one area. Mitotic activity in the retina was determined, as in the previous group of experiments, in complete specimens. In the first experimental series mice were twice injected subcutaneously with 0.5 mg of 5% glucose solution 3 and 5 hours before being killed. In the second experimental series starch (20 mg) was injected subcutaneously 3 hours before the animals were killed. In the third series insulin, in doses of 0.01, 0.05 and 0.1 milli-units or triprotamine zinc insulin in a dose of 0.1 milli-unit was injected. In the insulin experiments the mitotic activity was determined 3 hours after injection, and in the triprotamine zinc insulin experiments, 24 hours after injection. In the fourth experimental series the pancreatic islet cells were blocked by alloxan. This series of experiments was performed on white rats injected subcutaneously with 200-250 mg of alloxan per 1 kg body weight. As

a criterion of blockade of the islet cells we used histological examination of the pancreas and blood sugar estimations (by Hagedorn-Jensen's method). We produced experimental diabetes in 9 out of the 25 animals. On the 5th day after injection of allozan, the mitotic activity of these animals was examined. The sugar content at this time increased from 100-120 mg % in the control animals to 350-370 mg % in the experimental animals. Each experimental group had its own control group of animals. The results of these experiments are given in Tables 4 and 5.

TABLE 4

Mitotic Acitivity after Injections of Glucose and Insulin

Group of animals	Number animals	Number of mitoses	Phase coefficient
Control	10	52.7±9.2	2.6
Experimental, after injection of glucose	13	71.3±8.7	2.8
Control	13	76.0±15.3	5.8
Experimental, after injection insulin	19	84.0±13.2	6.5
Control	4	28.0±5.0	5.8
Experimental, after injection triprotamine zinc insulin	6	36.8±2.8	6.0

As can be seen from Tables 4 and 5, carbohydrate administration, insulin injection and blockade of the islet cells have no effect on mitotic activity. The widest variations do not transgress normal limits. Statistical treatment of the results by the Fisher-Student procedure shows that the variation between the experimental and control groups of mice was not significant.

TABLE 5

Mitotic Activity after Injections of Starch and Alloxan

Group animals	Number animals	Number mitoses and phase coefficient					
		Cornea		Skin		Tongue	
Control	5	140.0±8.6	1.96	11.6±5.3	3.68	32.6±8.2	6.38
Experimental, after injection of starch	5	146.0±23.0	2.0	12.6±3.5	3.0	42.6±10.2	6.0
Control	13	151.0±17.6	1.8	17.7±2.9	3.6	85.0±9.9	4.4
Experimental, after injection of alloxan	9	116.1±13.0	1.9	22.0±4.7	11.2	81.1±14.4	5.0

In the fifth experimental series we tried to explain the relation between the level of the blood sugar and the number of mitoses. For this purpose we determined the mitotic activity in the epithelium of the cornea, tongue and skin and the blood sugar in 17 normal white mice and 21 rats.

As can be seen from Table 6, we could show no association between the blood sugar and the number of mitoses. Experiments with white rats gave the same result.

TABLE 6

Mitotic Activity and Blood Sugar in White Mice

Experiment No.	Blood sugar (mg%)	Number of mitoses		
		Cornea	Skin	Tongue
120	94	125	18	7
121	98	21	7	12
131	98	80	10	7
123	102	4	21	7
128	119	94	13	6
108	120	40	15	12
114	134	34	25	7
105	134	111	35	8
127	133	73	11	10
122	140	78	—	5
111	141	10	14	12
125	144	81	3	8
124	145	61	12	13
129	147	11	—	13
132	147	46	18	6
126	149	40	18	7
133	153	116	29	11
	r+mr	-0.04 ± 0.24	$+0.29 \pm 0.24$	$+0.20 \pm 0.23$

As the same time we investigated the effect on cell division of sodium fluoride which is known to suppress the process of glyoclytic phosphorylation. Drops of sodium fluoride were instilled into the right eye $1\frac{1}{2}$ hours before the mice were killed. A single instillation of this compound in concentrations of 1 : 1000 and 2.5 : 1000 had no effect on mitotic activity in the corneal epithelium (10 experiments).

Analagous experiments were performed with malonates (1 : 1000-1 : 10,000; $1\frac{1}{2}$ hours before killing), which are known to block succino-dehydrogenase thereby interfering with carbohydrate oxidation. However, in these experiments too, no change in mitotic activity occurred in the cornea.

Thus, experiments with loading carbohydrate, injecting insulin, blocking the islet cells, injecting fluorides and malonates and determining the blood sugar and the mitotic activity gave straightforward results. Changes in carbohydrate metabolism, both stimulatory and inhibitory, brought about no change in mitotic activity of tissue. If carbohydrate metabolism is important in preparing the cell for division, this role is evidently secondary or is effected through other links of intermediate metabolism. Possibly cells possess sufficient stocks of high-energy phosphate compounds to allow division to take place in spite of disordered carbohydrate metabolism.

The experiments described show that nucleic acid metabolism is one of the essential biochemical processes concerned in cell division. Interference with nucleic acid synthesis leads to delay in the onset of cell division or to inhibition of mitosis at the prophase. Changes in carbohydrate metabolism have no appreciable effect on cell division.

SUMMARY

Disturbance of the process of phosphorylation by 2, 4-dinitrophenol, exclusion of desoxyribonuclease by adenine, derangement of the synthesis of nucleic acids by tripaflavine cause pronounced decrease of mitotic activity in the corneal, intestinal and tongue epithelium. Nucleic metabolism of a cell is one of the fundamental metabolic processes which is connected with cell division. Changes of the carbohydrate metabolism

(experiments with carbohydrate load, administration of insulin, block of the islet apparatus by alloxan, introduction of fluorides and malonates) in the direction of stimulation as well as in the direction of decrease of this metabolism do not result in the change of mitotic activity of the tissues.

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MORPHOLOGY AND PATHOMORPHOLOGY

EXPERIMENTAL DATA ON THE GENESIS OF CIRRHOSIS OF THE LIVER DUE TO DIETS DEFICIENT IN CHOLINE AND METHIONINE

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As a result of clinical and experimental investigations, considerable data have been collected relating to the importance of an alimentary factor in the genesis of disease of the liver. The study of experimental cirrhosis due to choline and methionine deficiency gives grounds for thinking that in human pathology conditions are possible which could lead to the onset of cirrhosis of the liver with a similar (or closely related) genesis [2].

However, in the problem of experimental cirrhosis due to dietary deficiency of sources of active methyl groups (choline, methionine), there is a number of problems on which opinions of investigators are conflicting. Some workers consider that cirrhosis is preceded by extensive necrosis of liver tissue* arising with or without fatty infiltration of the liver [7,9]. Meanwhile, experimental results show that the development of this type of necrosis in the liver is brought about by deficiency of S-containing amino acids but not of methyl groups, and that the consequences of this deficiency may to a certain extent be prevented by administration of vitamin E [8]. The question whether the presence of necrosis is really an essential condition for the development of cirrhosis remains unanswered.

Another question awaiting examination concerns the role of fatty infiltration. Some workers consider that the presence of prolonged fatty infiltration fulfils the conditions of development of cirrhosis and also that severe fatty infiltration is transformed into cirrhosis through a stage of "extracellular lipohepatosis", characterized by the appearance of masses of so-called fatty cysts. However, facts exist which demonstrate that the necessary conditions of development of cirrhosis are not fulfilled by fatty infiltration alone. For this reason investigation is necessary into the question whether additional factors besides the formation of fatty cysts do not precede experimental alimentary cirrhosis.

Finally, an explanation is also required of the nature of the resemblance between this particular form of experimental alimentary cirrhosis which we are examining and the cirrhosis observed in man. Some writers [7,3] consider that the pattern of experimental cirrhosis is close to that of cirrhosis in man, while others [11] think that the pathological conditions are only slightly comparable.

* This refers to extensive lesions of parts of a lobe or of whole lobes with necrosis of the epithelial and connective tissue parts of the organ. Dystrophic changes in the liver cells, undergoing fatty infiltration, in some cases terminating in their death, do not enter into the meaning of the expression "necrosis of liver" in this instance.

In order to answer these questions we performed the experiments described below.

EXPERIMENTAL METHODS

The experiments were performed on 70 male white rats weighing initially 40 to 90 g. We used the diet which was introduced by Daft [6] for production of cirrhosis. Some additions were made to the composition of the ration.

The dietary mixture which we used consisted of the following portions: casein (4%), lard (8%), corn starch (84.5%), salt mixture (3%) [10], cystine (0.5%). The animals received the following vitamins daily: thiamin (100γ), riboflavin (50γ), pyridoxin (20γ), calcium pantothenate (20γ), nicotinic acid (1 mg), vitamin A (20 international units), vitamin D (8 international units). Once a week each rat was given 3 mg of vitamin E. Inclusion of vitamin E in the diet was with the aim of preventing the development of necrosis of the liver as mentioned above.

We also had the intention of reproducing alimentary cirrhosis under conditions pathogenetically relatively close to those in which a similar type of cirrhosis may arise in man. Investigations showed that deficiency of protein in the diet involves the development of endogenous choline insufficiency [4]; for this reason we included in the diet a small quantity of protein. However, in view of the fact that in small quantity casein is insufficient on account of its lack of S-containing amino acids [12], we included cystine in the diet. Tryptophan deficiency could arise; we compensated for this to some extent by the addition of nicotinic acid. The increased amount of thiamin in the diet was on account of its high carbohydrate content.

In order to study the successive phases of the changes in the liver, we periodically performed biopsy. For this purpose, under ether anesthesia we made a small incision in the epigastric region and delivered the left lobe of the liver. We excised a small piece measuring 0.5 cm^2; the omentum was applied to the wound in the liver to control hemorrhage. The peritoneum and muscular wall were closed with continuous sutures and the skin with interrupted sutures. In not one out of 30 cases of biopsy, including some repeated operations, did the animal die. In addition, some organs (liver, lungs, kidneys) of animals killed at various stages of the experiment were examined under the microscope.

Histological preparations (fixation in 10% formalin, imbedding in paraffin and gelatin) were stained with Sudan III by Goldman's method, hematoxylineosin, picrofuchsin mixture, carbolfuchsin (ceroid) and were also impregnated by the Tibor-Pan-Snesarev method.

The maximal length of time spent by the animals in the experiment was 16 months.

EXPERIMENTAL RESULTS

In the first 3 months was noticed the development of severe fatty infiltration of the liver. The data relating to this period are from specimens obtained from 28 animals; in 18 of them biopsy was carried out.

The first biopsy was performed on the 3rd day of the experiment. Macroscopically the liver was clay colored; microscopically small droplets of fat were seen in the peripheral portions of the lobules. At the end of the first month, these portions were in a state of severe fatty infiltration, while the protoplasm of the liver cells close to the central veins contained only small droplets of fat. During the following 2 months the severe fatty infiltration spread to the central zones of the lobules and acquired a diffuse character. At this time numerous fat emboli (Fig. 1) began to appear in the capillaries of the lungs; in individual cases fat was discovered in the lumen of the small intrahepatic bile ducts. The deposition of fat in the vascular and biliary systems was connected with the appearance in the liver of so-called fatty cysts, the formation of which and the possibility of rupture into the vascular and biliary systems were described by W. Hartroft [14]. In addition to fatty infiltration were also seen necrosing liver cells with disappearance of the membrane and dissolution of the nucleus. At this period also was formed the pigment ceroid, staining a crimson color with carbolfuchsin. Pigment granules were seen in the connective tissue of Glisson's capsule and along the course of the hepatic veins.

The state of the liver in the next period of the experiments – between the 4th and 12th months – was also studied by us in 28 animals. Without reducing its size, the liver became superficially much more granular.

Microsopically, beginning on the 5th-6th month, the quantity of visible fat in the majority of cases diminished sharply. This process was accompanied by the appearance of numerous diffusely scattered foci of regeneration (Fig. 1. b), and in the protoplasm of the newly formed liver cells no fat could be demonstrated. Signs of marked cirrhosis were present in the 10th-12th months.

In the next period of observation – between the 12th and 16th months of the experiment – changes in the liver were studied in 14 rats; five of them had a preliminary biopsy. In the majority of the animals of this group, the pattern of cirrhosis of the liver had developed, consisting of a combination of dystrophic and regenerative changes, diffuse sclerosis and complete reorganization of the normal structure of the liver.

However, in individual animals (rats Nos. 35, 56 and 61) the pattern of severe fatty infiltration without sign of cirrhosis continued to be maintained. In histological preparations of the liver in these cases were found small and larger (up to 42 μ in gelatin) fatty cysts.

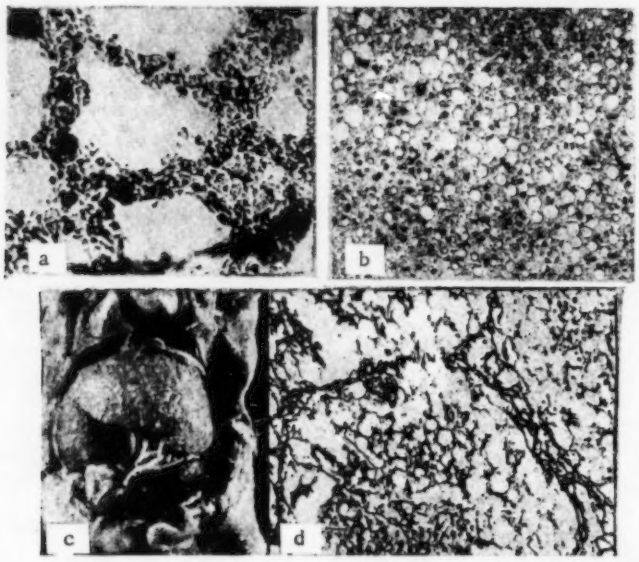

Fig. 1. Tissue changes in experimental cirrhosis in rats. a) Fat emboli in the interalveolar capillaries of the lungs on the 78th day of the experiment. Rat No. 25. Stained with Sudan III and hematoxylin. Magnification 80; b) small foci of regeneration against a background of fatty infiltration of liver cells still present on the 171st day of the experiment. Rat No. 41. Stained with hematoxylineosin. Magnification 80; c) cirrhosis of the liver (naked eye specimen); d) structure of liver tissue 16 months after the experiment. Impregnation by Tibor-Pan-Snesarev method. Magnification 100.

The total number of these cysts was not large and intracellular fat was more prevalent. In the zone corresponding to the most severe fatty infiltration, i. e., the periportal zone, thickening and coarsening of the argyrophilic fibers was noticed (Fig. 2). By comparison with cases where development of cirrhosis had occurred, in the liver of these animals attention was drawn to the weakly-expressed process of regeneration of the parenchyma. In these cases regeneration did not lead to the production of a large number of nodules of newly-forming parenchyma; it consisted of the disorderly new formation of small and large cells, sometimes of multinucleated liver cells, diffusely scattered in the parenchyma and, as a rule, also affected by fatty infiltration. In spite of the severe degree of the latter and the absence of a trabeculo-lobular structure pattern,

no reorganization of the vascular tree could be seen in the liver; there was no coarse, diffuse sclerosis. All these circumstances do not allow the changes observed in these animals on the 12th-16th months to be regarded as cirrhosis.

Necrosis was not observed, neither in early stages nor in the period of maximal development of fatty infiltration, even if this was maintained for 12 to 16 months.

Thus the experiments showed that the animals developed severe fatty infiltration of the liver, and against this background the pattern of cirrhosis took shape. This cirrhosis appeared to be a combination of dystrophy, regeneration, diffuse sclerosis and reorganization, i, e. signs by which the true cirrhosis of the liver in man is characterized [1].

Earlier we pointed out the opinion of some workers, that the development of cirrhosis is preceded by necrosis of the liver. In our experiments we avoided necrosis, giving the animals vitamin E, and in every case cirrhosis developed without preceding widespread necrosis. As a result we may consider that such necrosis is not a necessary condition of development of this type of cirrhosis of the liver.

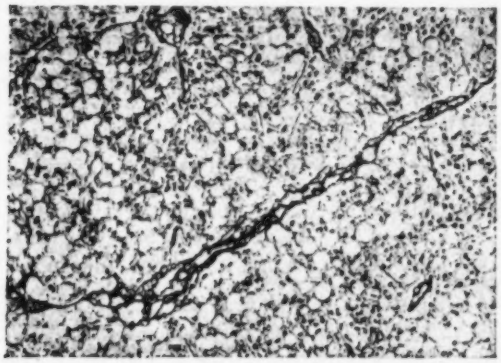

Fig. 2. Coarse argyrophilic fibers in the periportal zones of the liver of rats with experimental cirrhosis of the liver.
Duration of the experiment 14½ months. Impregnation by the Tibor-Pan-Snesarev method. Magnification 80.

Scrutiny of the successive microscopic patterns of change in the liver enables one further conclusion to be made. Some workers, studying the phases preceding the development of cirrhosis, have fixed their attention on the fatty infiltration of the liver, and have connected cirrhosis genetically with later transformations of the fatty cysts. From this point of view, in our experiments we must note cases in which, in spite of the long duration of the experiment, with severe fatty infiltration and the presence of fatty cysts, cirrhosis did not develop (in 3 out of 14 rats in experiments lasting from 12-16 months). It is very important that in these cases we did not observe any marked degree of regeneration. From these findings, we consider that in addition to the formation of fatty cysts, in the production of cirrhosis as studied in our experiments an essential role is played by regenerative processes.

The results of our experiments draw attention to one further fact. In the experimental rats we did not find cirrhosis before 10-12 months after the beginning of the experiments, while according to Canadian and some American workers cirrhosis develops in the animals in the 4th month of the experiments. This may probably be explained by the fact that the choline and methionine requirements of rats of different strains may vary [5].

SUMMARY

Cirrhosis of the liver, which occurs as a result of prolonged deficiency of choline and methionine in food, developed in those experimental animals in which intensive process of regeneration of the parenchyma took place together with considerable fatty infiltration. Necrosis of the liver is not a required condition in development of the cirrhosis of the liver which was studied.

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THE EFFECT OF PENICILLIN ON THE BLOOD SYSTEM

THE EFFECT OF PENICILLIN ON THE COMPOSITION OF THE PERIPHERAL BLOOD AND BONE MARROW IN MAN AND ANIMALS IN NORMAL HEALTH

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The introduction and widespread use of antibiotics in clinical practice has called for careful investigation of changes which may arise in various systems and organs. This investigation showed that in certain cases phenomena may be observed which must be assessed as side effects in relation to the therapeutic value of the antibiotics.

Changes in the blood system by the action of antibiotics have been studied by several workers, but their findings show considerable disagreement [2,3,5]. In individual cases, antibiotics may even cause an aplastic condition of the bone marrow [6,7]; so far as penicillin is concerned the majority of workers mention only the development of eosinophilia [1,4] in patients receiving this preparation. The mode of production of this eosinophilia has not yet been fully explained, since the problem of the action of penicillin on the blood system of man and animals in normal health has not yet been adequately studied. Such a study would enable a number of doubtful problems concerning the mechanism of development of these changes to be cleared up, since in this case the effect of penicillin would be studied without the ever present effects of the desease itself.

We studied the changes in the composition of the peripheral blood in 10 healthy human subjects and 18 animals (10 rabbits and 8 cats). In the 8 cats, in addition to the peripheral blood bone marrow hemopoiesis was also studied.

EXPERIMENTAL METHODS

Penicillin was injected every 3 hours, in a daily dose of 320,000 to 800,000 units, into persons prepared for plastic operations. The injections lasted for 7 to 35 days. The blood was examined 2-3 times before and 4 times during penicillin therapy. Hemoglobin estimation, erythrocyte and leucocyte counts and the leucocytic formula were determined; films were stained by the Romanowsky-Giemsa method, counting 400 cells.

Analagous investigations were made on 10 rabbits and 8 cats. Penicillin was injected intramuscularly in a dose of 50,000 units every 4 hours (300,000 units daily). The total dose of the antibiotic in the course varied between 2,100,000 and 18,600,000 units. Blood was taken from an incision in the ear one hour after injection of penicillin, in turn; besides estimation of the hemoglobin, erythrocytes, leucocytes and leucocytic formula the reticulocyte count was also determined in the blood of the rabbits. In addition to the study of the effect of prolonged administration of the antibiotic, changes in the composition of the blood of cats arising during the 3 hours following injection were investigated. As a basis for comparison the initial state of the blood was

examined for 2-3 weeks before the experiments (usually every other day).

EXPERIMENTAL RESULTS

The human subjects were carefully examined before penicillin therapy, and none of them showed any inflammatory condition, raised temperature or deviation of their initial blood composition from normal.

The table shows the average results of the changes in hemoglobin content and in the erythrocyte count before and after penicillin treatment in all the subjects.

Changes in the Hemoglobin Content (Sahli) and the Erythrocyte Count in Human Subjects due to Penicillin, in 1 mm^3

Subject	Before penicillin admin.		Completion penicillin admin.	
	Hemoglobin	Erythrocytes	Hemoglobin	Erythrocytes
M-ya	78	4 300 000	75	4 500 000
Ch-a	62	3 800 000	65	4 000 000
Sh	75	4 160 000	76	3 950 000
S-a	82	4 200 000	77	4 520 000
F-a	63	3 580 000	68	4 200 000
P-a	68	3 800 000	66	3 900 000
K-r	72	4 800 000	76	4 700 000
Kh-a	73	4 550 000	70	4 600 000
Z-ch	76	4 280 000	70	4 140 000
D-a	65	3 960 000	69	4 000 000

The table shows that no significant changes occurred in the hemoglobin content and erythrocyte count. Slight changes occurred in the leucocyte count; thus in 5 persons it increased (at the most by 3000 per 1 mm^3), in 4 it was unchanged and in only one did it fall on the 4th day of penicillin administration to 2800 per 1 mm^3, however on the 7th day it had risen to 6400. Regarding the eosinophilia observed in some patients, we paid great attention to determining the number of eosinophils; However, we did not obtain any convincing results. In 8 out of the 10 subjects the eosinophil content did not exceed 3%, and in only one of them did we find a brief (for one day) rise in the eosinophil content to 9% subsequently falling to 2%; finally, in the last subject it fell from 4 to 1-3%. Calculation of the absolute value of eosinophils did not introduce any appreciable correction to the results obtained. Thus, in all the subjects penicillin administration produced no appreciable change in the composition of the peripheral blood.

The rabbit experiments showed that prolonged administration of antibiotics produced no appreciable change in the composition of the blood of the animals. Thus, variations in the leucocyte count between 6000 and 10,000 per 1 mm^3 did not exceed arbitrary limits. The same result was obtained with the cats. The hemoglobin content varied little, and in no case more than it did before administration of the antibiotic (within limits of 10-12 Sahli units).

The results of the reticulocyte counts, shown in Fig. 1, indicate that the changes in this index were irregular. It is true that in 4 rabbits there was some degree of reticulocytosis (from 0.6 to 3.2%), but these variations do not transgress the normally accepted limits.

Figure 1 illustrates the changes in the content of eosinophils (the estimation of eosinophils in rabbits is made difficult by the presence of pseudo-eosinophils, but it is perfectly possible). It was shown that in 6 animals, the number of eosinophils varied during the period of penicillin administration between 0-124 cells per 1 mm^3 (expressed in absolute figures). In 3 rabbits a rise in this figure to 204 cells was noted on the 9th

day of administration, and in 1-196 on the 32nd day of penicillin administration. Expressed as percentages, these variations were from 0-2%. No changes were found in the other components of the leucocytic formula.

Quite similar results were obtained from the cat experiments. Investigation of the "acute" changes, i. e. those occurring within 3 hours of the injections showed constantly an increase in the leucocyte count (within limits of 3000-6000 cells per 1 mm^3) which appeared only during the first hour. Variations in the eosinophil content were slight, within limits of 0-4%.

Since the blood changes in the healthy human subjects and animals caused by penicillin were insignificant, whereas clinical experience, including our own investigations, speaks of the development of eosinophilia in some such cases, we studied the state of bone marrow hemopoiesis in 8 animals (cats). Bone marrow was obtained by puncture of the distal epiphysis of the femur of these animals; besides studying the usual myelogram we also investigated the course of maturation of the eosinophils, making it possible to judge the effect of penicillin on eosinophilic growth in the bone marrow.

Analysis of the myelograms showed a tendency towards an increased content of erythroblasts, which led to a small reduction in the relation of leucoblastic and erythroblastic growth (Fig. 2). The observed in-

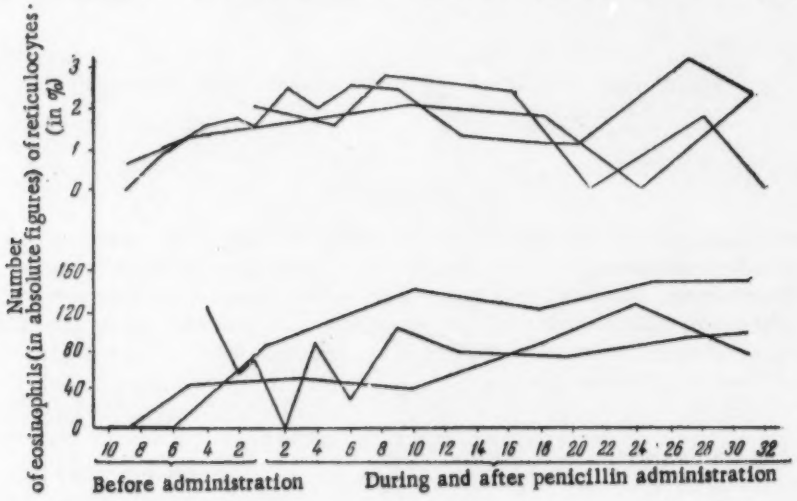

Fig. 1. Changes in the eosinophil and reticulocyte counts of rabbits with administration of penicillin.

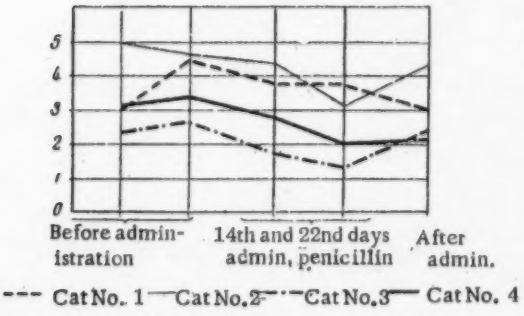

Fig. 2. Changes in the relation between leucoblastic and erythroblastic growth with administration of penicillin to cats.

crease in the number of erythroblasts affected all forms, but mainly the oxyphils. In Fig. 3 are shown changes in the maturation curve and the number of eosinophils in the bone marrow arising as a result of penicillin. These were most clearly shown in cat No. 4 in which there was a sharp increase in the content of more mature forms (eosinophilic metamyelocytes and polymorphonuclear cells). In two other animals similar changes were found, but to a lesser degree. In cat No. 1 there was a rise in the number of eosinophilic myelocytes and a fall in the content of more mature forms. Discontinuation of the penicillin in all cases led to restoration of the original relationship within 7-9 days.

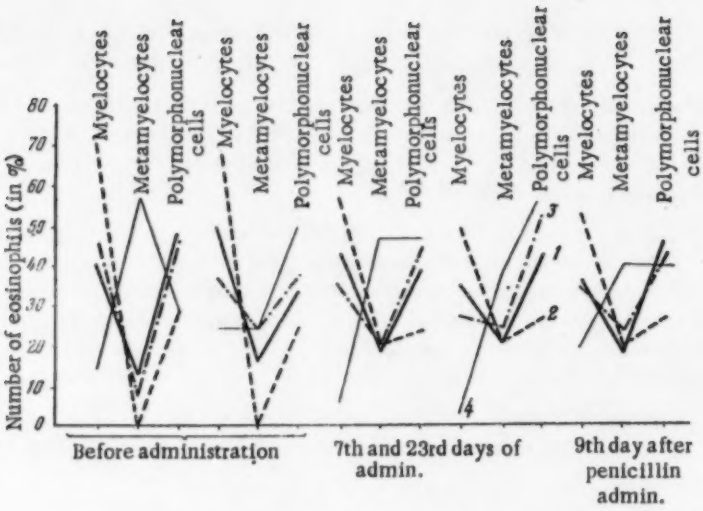

Fig. 3. Maturation of eosinophils with administration of penicillin to cats (1, 2, 3, 4).

SUMMARY

No marked changes have been noted in the peripheral blood of healthy humans injected with penicillin for 1-5 weeks. The same effect was noted in rabbits and cats. Some increase in the number of reticulocytes did not exceed normal values. Studies of bone marrow hemopoiesis have revealed a tendency to an increased number of erythroblasts, mainly of the oxyphilic group, and a rise in the number of eosinophils with their more early maturation. No such phenomena were noted in the peripheral blood. With cessation of penicillin injections initial states were restored in all cases.

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December, 1957

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RUSSIAN JOURNALS FREQUENTLY CITED
[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiia	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiia	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Embriol.	Arkhiv Anatomii Gistologii i Embriologii	Archives of Anatomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiia	Biokhimiia	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiia Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Biull. Eksptl. Biol. i Med.	Biulleten Eksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Biull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Biulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Eksptl. Khirurg.	Eksperimentalnaia Khirurgiia	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiia i Toksikologiia	Pharmacology and Toxicology
Farmatsiia	Farmatsiia	Pharmacy
Fiziol. Rastenii	Fiziologiia Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenova Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariia	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiia Akademii Nauk SSSR, Seriia Biologicheskaia	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiia Tikhookeanskogo N. I. Instituta Rybnogo Khoziaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiia	Khirurgiia	Surgery
Klin. Med.	Klinicheskaia Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo (po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaia Parazitologiia i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaia Radiologiia	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiia	Mikrobiologiia	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhiat. i Psikhogig.	Nevropatologiia, Psikhiatriia i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediia, Travmatologiia i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriia	Pediatriia	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Endokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniia Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaia Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journals
Stomatologiia	Stomatologiia	Stomatology
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriia	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics

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Abbreviation	Journal	Translation
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiia	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiia	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiia, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoiuz. Gidrobiol. Obshchestva	Trudy Vsesoiuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoiuz. Inst. Eksptl. Med.	Trudy Vsesoiuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiia	Urologiia	Urology
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Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiia	Progress in Contemporary Biology
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Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
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Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaia Laboratoriia	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiia	Journal of General Biology
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